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UNIVERSIDADE DO PORTO

AGE-RELATED CHANGES OF THE PELVIC FLOOR LIGAMENTS, A BIOMECHANICAL STUDY

FRANCISCO BULHOSA DA ROCHA PEREIRA
DISSERTAÇÃO DE MESTRADO APRESENTADA
À FACULDADE DE ENGENHARIA DA UNIVERSIDADE DO PORTO EM
ENGENHARIA BIOMÉDICA

U.PORTO
FEUP FACULDADE DE ENGENHARIA UNIVERSIDADE DO PORTO

MEB - MESTRADO EM ENGENHARIA BIOMÉDICA | **2014/2015**

A Dissertação intitulada

"Age-related Changes of the Pelvic Floor Ligaments, a Biomechanical Study"

foi aprovada em provas realizadas em 14-07-2015

o júri

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Age-related Changes of the Pelvic Floor Ligaments, a Biomechanical Study

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PRELIMINARY VERSION

Master's Dissertation

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June 2015

A todos aqueles que me aturaram até chegar aqui…

Aos meus irmãos pelos cabelos brancos…

E depois aos meus pais e à patroa…

“We all change, when you think about it, we're all different people; all through our lives, and that's okay, that's good you've gotta keep moving, so long as you remember all the people that you used to be…”

The Doctor

Resumo

O pavimento pélvico feminino está localizado na extremidade inferior da cavidade abdominal desempenhando funções passivas e activas a fisionomia das vísceras pélvicas. Trata-se de um conjunto de estruturas intimamente ligadas, sendo que a falha de um dos componentes pode levar ao colapso do sistema. As desordens do pavimento pélvico vão então comprometer o correcto funcionamento deste sistema. Incluem-se todas as patologias que possam afectar o pavimento pélvico. Neste trabalho apenas são estudadas a incontinência urinária e fecal e o prolapso pélvico, sendo o ultimo um ponto central de discussão no plano deste trabalho.

As causas do aparecimento das desordens são várias, estando o envelhecimento em destaque na falência da correcta funcionalidade do pavimento pélvico. Os mecanismos que levam à origem destas falhas ainda não são claramente percebidos e é neste panorama que este trabalho se insere.

Os ligamentos pélvicos são responsáveis pelo suporte e auxilio da actividade dos órgãos pélvicos. A falência destes pode levar ao aparecimento de patologias afectando o funcionamento dos órgãos.

Sabe-se que os ligamentos têm um comportamento mecânico não linear hiperelastico, tratando-se de tecidos anisotrópicos com propriedades tempo- e histórico-dependentes (viscoelástico).

O estudo do pavimento pélvico passa pela caracterização biomecânica dos ligamentos pélvicos, através de testes de tensão uniaxiais. Estes vão permitir estabelecer um comportamento típico dos ligamentos para no futuro ser possível adaptar modelos hiperelásticos que mimetizem o comportamento destes tecidos.

Este trabalho continua a decorrer, sendo que os dados apresentados dizem respeito aos testes efectuados até à data. Apesar de não ser possível tirar conclusões, os resultados tem vindo a demonstrar o comportamento esperado.

Abstract

The female pelvic floor is located at the bottom of the abdominal cavity, taking part actively and passively in the physiological activity of the pelvic viscera. It is a set of structures intimately connected; hence the loss of function in one area can lead to the failure of the whole system. Pelvic floor disorders will compromise the activity of this system. Those are all the pathologies affecting the pelvic floor. In this work three will be considered, urinary and faecal incontinence and pelvic prolapse, being the later a central discussion point in this research.

There are several causes for pelvic floor disorders, being aging closely related to the failure of the physiology of the pelvic floor. The mechanisms which lead to the failure are yet to be clearly understood and this is where this project may give its contribution.

Pelvic ligaments are responsible for the support and aid in activity of the pelvic organs. The failure of these leads to pelvic pathologies affecting the performance of those organs.

Ligaments are known to have non-linear hyperelastic behaviour, being anisotropic tissues with time- and history-dependent properties (viscoelastic).

The study of these structures is focused in the biomechanical characterization of the pelvic ligaments through uniaxial tension tests. These will allow to establish the typical mechanical behaviour so that in the future hyperelastic models can be adapted to mimic the behaviour of these tissues.

This project is still in progress, and the results here presented refer to the data collected so far. Even though conclusions cannot be clearly stated, the results tend to follow the expected behaviour.

Agradecimentos

Tenho de começar por deixar uma palavra de agradecimento a algumas pessoas, visto que sem elas nada disto seria possível…

Começo por agradecer ao meu coordenador, Professor Doutor Pedro Martins, por toda a disponibilidade, sinceridade, paciência e partilha que teve comigo nestes últimos tempos… Ao meu co-orientador, Professor Doutor Renato Natal, à Nilza Raimão, à Professora Dra. Teresa Mascarenhas… À Dra. Sara Oliveira e ao Dr. Pedro Pinto, médicos internos no Hospital São, pela cooperação e disponibilidade.

Aos burdadeiros do Palacio cor-de-rosa, Bio continuará a ser a minha casa… Aos de espinho que por lá andam, ou que de lá saíram, aos recentes e aos de longa data… Aos de longe e aos que já fugiram de cá… Por muitas voltas que venha a dar, sei onde posso voltar!

A toda a minha família e ao resto que vêm emprestado… Aos meus pais, Mário Augusto e Ana Paulinha, por TUDO! Aos meus irmãos, Rodrigo e Rita, sou um irmão babado! À avó Laura e avô Tono-Agusto, à tia Guida e tio Luís, à melga da Sofia e ao “Tiaguinho”, ao Cacu Miguel e à Dani, não há forma de agradecer todo o amor e carinho… Por fim à minha patroa porque envelhecer é obrigatório mas crescer é opcional!

…

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Nomenclature

List of acronyms

PFD	Pelvic Floor Disorder
POP	Pelvic Organ Prolapse
UI	Urinary Incontinence
FI	Faecal Incontinence
PD	Pelvic Diaphragm
LA	Levator Ani
CM	Coccyxgeus Muscle
ICM	Iliococcygeus muscle
PCM	Pubococcygeus muscle
TALA	Tendinous Arch of Levator Ani
UD	Urogenital diaphragm
PRL	Puborectalis ligament
EAS	External Anal Sphincter
PD	Pelvic Diaphragm
PM	Perineal Membrane
PB	Perineal Body
EF	Endopelvic fascia
FT	Fascial Tissue
PL	Pelvic Ligaments
USL	Uterosacral Ligaments
CL	Cardinal Ligament
PVL	Pubovesical ligament
PRL	Puborectalis ligament
ACL	Anococcygeal ligament
TAPF	Tendinous Arch of Pelvic Fascia
PUL	Pubourethral Ligament
EUL	External Urethral Ligament
EUM	External Urethral Meatus
DM	Detrusor Muscle

SUI	Stress Urinary Incontinence
UUI	Urge Urinary Incontinence
MUI	Mixed Urinary Incontinence
MRI	Magnetic Resonance Imaging
MUS	Medical Ultrasonography

List of symbols

Ψ	Strain.energy function
Δl	elongation
σ	stress
A_0	initial transverse area
A_1	deformed transverse area
l_0	initial length
l_1	deformed length
λ	stretch
ε	deformation
E	Young's modulus
Es	secant modulus
Et	tangent modulus
σ_y	yielding stress
σ_{max}	maximum stress
λ_U	ultimate stretch
U_s	energy density

Chapter 1.

Introduction

The pelvic floor is a complex and dynamic system, with multiple functions. The pelvic floor soft tissues not only act passively in the activity of the organism, supporting the pelvic viscera, but also participate actively in the functionality of the pelvic organs. The condensation of the fascial sheet, covering the pelvic muscles and organs, originates the pelvic ligaments anchoring the pelvic organs to the bony pelvis [1]. As it is an interconnected structure, the failure of one part will lead to the failure of the whole system and hence pelvic floor dysfunctions.

Pelvic floor dysfunction (PFD) is a term used to define the pathologies affecting the pelvic floor, and 31% to 40% of parous women are affected by some kind of dysfunction [2]. The dysfunctions considered in this report are: pelvic organ prolapse (POP), urinary incontinence (UI) and faecal incontinence (FI). PFD are usually related with age, since older women tend to be more susceptible to these types of problems. Aging and childbirth are considered the greatest risk factors for PFD [3]. Apart from this, their origin is not very clear yet, since their development may also be attributed to diverse trauma sustained by the tissues. These dysfunctions usually require a multidisciplinary approach for their correct assessment [3].

The dysfunctions affecting the pelvic floor are many times related to a loss of support of the organs or by weakened endopelvic connective tissue and floor musculature. POP refers to the sliding out of place of the pelvic organs. IU is the involuntary loss of urine and is majorly related with problems associated with the urethra. As for FI, it is defined as the involuntary loss of faeces and is usually related to dysfunctions on the anal sphincters [4, 5].

The diagnosis of PFD can be performed by physical examinations, urodynamic tests (in the case of UI) and several imagiology technics. As for treating the disorders, the conservative treatments consist mainly on muscle reinforcement, pharmacological treatments and use of pessaries (in the case of POP). As these only limit the progression of the pathology, surgical treatment is required. The surgical alternatives for PFD usually try to provide support to the pelvic structures. It is estimated that the lifetime risk of undergoing pelvic surgery is 11% [6].

The impact of aging on tissues in general, is an important subject and concerning pelvic soft tissue, and is one of the goals of this work. The process of aging is related to PFD since its one of the major risk factors. Aging affects different tissues in multiple ways. In the case of ligaments, it has effects on their molecular organization, resulting in an overall degradation of the tissue. Ligaments are fibrous and highly organized tissue, with collagen fibres playing a major role in its characteristics [7]. This organization is responsible for the anisotropic response of ligaments [8]. As the tissue ages the interconnection between collagen fibres will be affected, as well as its quality. The effects of aging are one of the main focus in this work.

The study of the pelvic floor must also include the study of the biomechanical principles by which the tissues will be examined. Soft tissues are known to have hyperelastic behaviour. To model their behaviour correctly, specific mathematical functions are used (constitutive models). These functions will later on be compared to the results obtained from tension tests.

The lack of information regarding pelvic ligaments behaviour, and how this behaviour is affected by aging, lead to this study. To assess the mechanical characteristics and degradation of pelvic ligaments may allow to assess pelvic floor dysfunctions, focusing on the origin of the problem. The highly oriented fibrous structure of tissues, such has ligaments and tendons, affects the mechanical behaviour of the tissue matrix, hence the importance of to study ligaments. Therefore, the objective of this work is to study the influence of aging on pelvic ligaments, as well as the influence of the fibre direction.

Chapter 2.

Female Pelvic Anatomy

2.1. Introduction

To fully understand the problems affecting the pelvic floor we must start by having a clear picture over the anatomy of this area. The pelvic cavity can be defined by the viscera present between the pelvic peritoneum and the vulvar skin [9]. The pelvic floor can be seen as a complex and inter-dependent set of structures which includes muscles, ligaments, fascia and other connective tissue. It not only supports the pelvic organs, but also aids in their function, maintaining continence and facilitating micturition and evacuation.

Although there is a good knowledge over the anatomy of the region, the biomechanical functions of the pelvic floor are not well understood and it is essential for knowledge to be continuously evolving [10].

2.2. Bony Pelvis

The skeleton acts as the body's deposit for calcium, supports and transports the body weight, protects the inner organs, as well as, providing fixation points for other soft tissues. In terms of bones, the pelvic cavity is limited by the pelvic bones, sagitally and anteriorly, and by the coccyx and sacrum posteriorly.

The pelvic bone is formed by the hip bone, the sacrum and the coccyx. The hip bone is divided into the ilium, ischium and pubis, which is fused with the coccyx (posterior) and by the pubic symphysis (anterior) [9, 11].

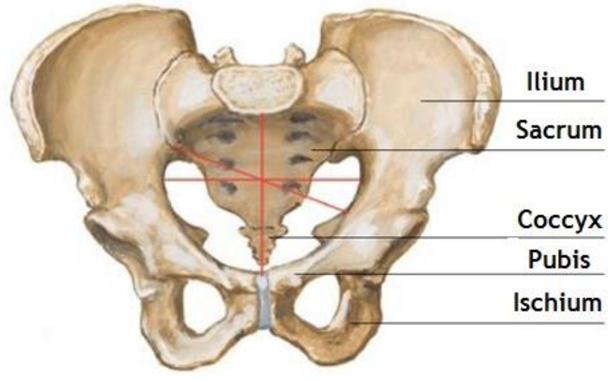


Figure 2. 1- Anterior view of the female pelvic bone adpted from Netter[12]

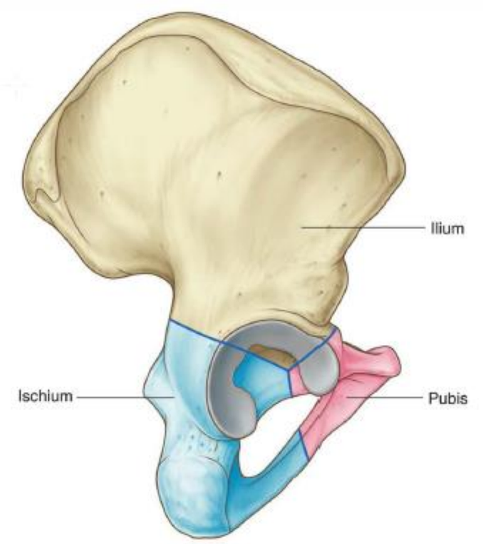


Figure 2. 2 – Lateral view of the hip bone adapted from Drake et al. [10].

2.2.1. True pelvis

The true pelvis has an inverted dome-like shape and is related to the inferior parts of the pelvic bones. It is consists of an inlet, a wall, and an outlet. It separates the pelvic cavity from the perineum and it houses the pelvic organs [9, 4].

<u>Pelvic inlet</u>

The pelvic inlet can be is referred as the circular opening separating the abdominal cavity from pelvic cavity. It is through the pelvic inlet that structures pass and this structure is surrounded by bones and joints.

<u>Pelvic wall</u>

The pelvic wall consists of the pelvic bones limiting the lesser pelvis, two ligaments and two muscles. The support of the pelvic viscera will be later on addressed, but it is on the pelvic wall that the support relies, though connective tissue attachments. The pelvic viscera includes the urinary and reproductive system, as well as, part of the gastrointestinal system.

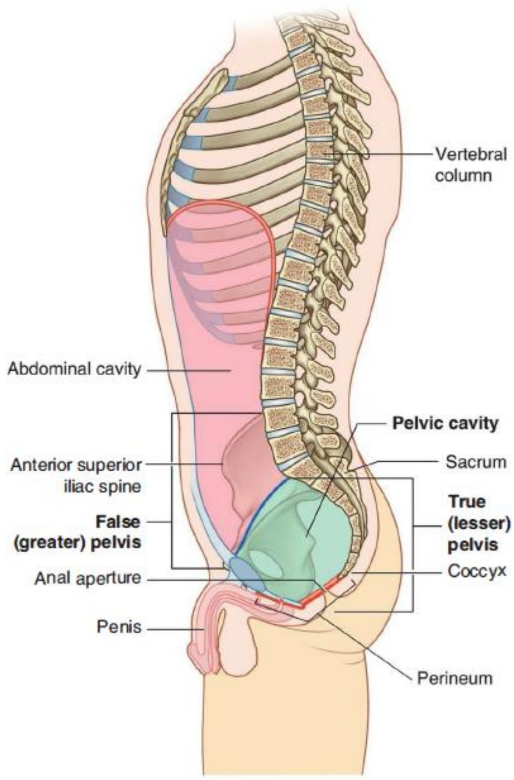


Figure 2. 3 - Representation of the pelvic and abdominal cavity; Differentiation between false pelvis and true pelvis adapted from Drake et al. [10].

Ligaments

The sacrospinous and sacrotuberous ligaments are part of the lateral pelvic wall. These ligaments not only help define the apertures of the pelvic cavity, but also act on the stabilization of the sacrum on the pelvic bones, tilting the inferior aspect of the sacrum

Muscles

The muscles which form the pelvic wall are the obturator internus and the piriformis muscles. These muscles are essential to the lower limb, being responsible for the lateral rotation of the extended hip joint.

Apertures

The pelvic wall has three major apertures: the obturator canal, the greater sciatic foramen and the lesser sciatic foramen.

<u>Pelvic Outlet</u>

The pelvic outlet forms a diamond shaped line, with its anterior part defined by bones and the pubic symphysis, and posterior part defined by ligaments and the coccyx. The anterior part is also named the pubic arch. A wider outlet can be beneficial to childbirth but is also a predisposition to pelvic floor dysfunctions.

2.3. Muscle of the Pelvic Floor

The main groups of muscles present in the pelvic floor are the pevic diaphragm (PD), the perineal membrane (PM), also known as urogenital diaphragm, and the perineal body [8, 12].

<u>Pelvic Diaphragm (PD)</u>

The pelvic diaphragm is also referred as the levator plate. It is formed by the levator ani (LA) and the coccygeus muscle (CM) form the PD, which combined with fascial tissue (FT) forms a dome like structure, acting as a base for the pelvic organs[8, 13, 14].

The constant tone from these muscles, formed by type I strained muscle fibers, prevents the ligaments from becoming overstretched and damaged[3].

The LA is divided into the iliococcygeus (ICM), the pubococcygeus (PCM) and the puborectalis muscle (PRM), depending on the orientation and origin. The ICM and PRM originate from the ischial spine, the tendinous arch of levator ani (TALA) and the pubic bone, and as for the PCM, it is inserted in the anterior half of the TALA and the pubic bone. The “U” shape around the anorectum is formed mainly by the PRM [8, 13, 14].

The CM is inserted in the tip of the ischial spine, supporting the posterior part of the PD. The muscular fibers extend to the coccys-saccrum complex. This muscle is the homologous for a tail muscle in other species [8, 13, 14].

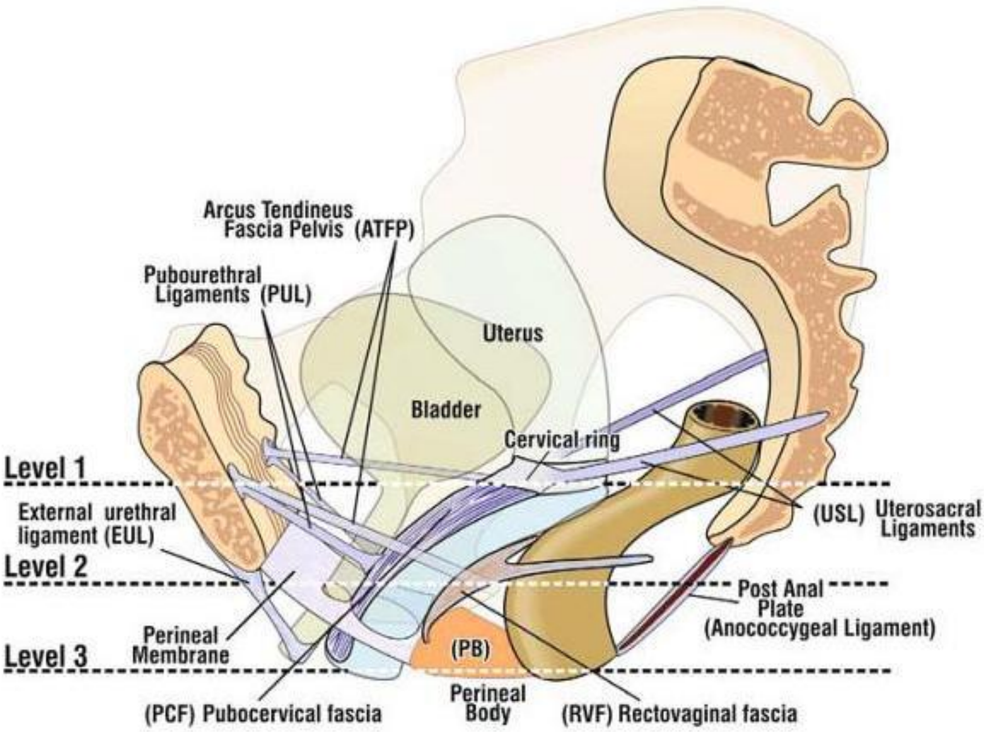


Figure 2. 4 - Representation of connective tissue support levels and their relation with the pelvic organs and bony pelvis [13].

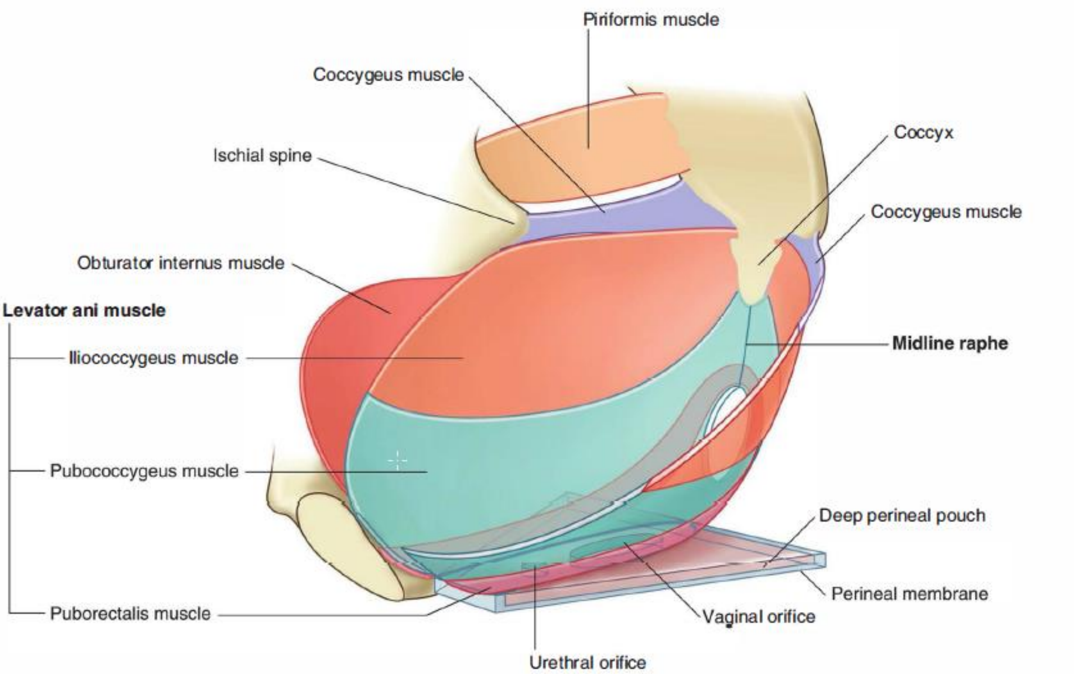


Figure 2. 5 - Schematic representation of the pelvic muscles adapted from Drake et al. [10].

These muscles play an active role in urinary, faecal and sexual activity. During child birth, through vaginal delivery, the LAM is submitted to extreme stresses, which can lead to damage, often near the pubic bone insertion [3].

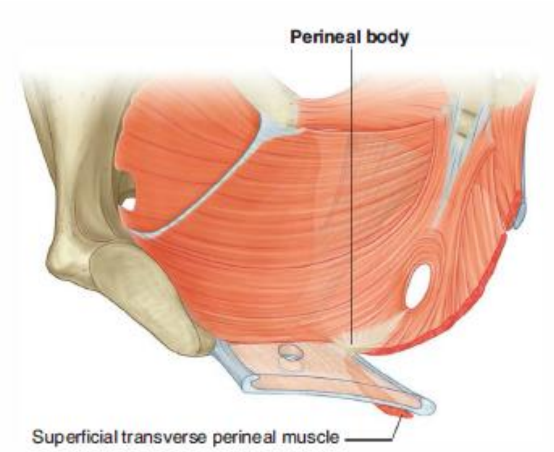


Figure 2. 6 - Representation of the perineal body and its localization in the pelvic floor adapted from Drake et al. [10].

Perineal membrane (PM)

The perineal membrane, also known as the urogenital diaphragm, is located right below the PD (PD), crossed by the urethra and vagina. It is formed by the ischiocavernousus muscle, the bulbospongiosus muscle and the transverse perineal, fusing with the external anal sphincter (EAS) in the perineal body (PB) [3, 9, 11]

Perineal body (PB)

The PB is located between the EAS and the vulva. It is an intersection point for several other structures, therefore, important for all those involved. The longitudinal muscle of the anorectum, the PCM, PM and EAS are the structures that give shape to the PM [13-15]

2.4. Connective tissue: Fascia and Pelvic ligaments

The several types of tissue found in the pelvic viscera are essential to the dynamics of the pelvic floor in their own way. This being said, it is known that FT is relatively stiff, compared to other types of tissues. This variety of properties ensures a functional pelvic floor. With childbirth or natural aging these structures can sustain damage or degradation [15, 16]

These tissues can be divided into three levels of support; in a clinical perspective the division will aid classifying a prolapse (Figure 2.4). This dysfunction can be classified as apical prolapse, cystoceles and rectoceles. This division is a classification of the area of the pelvic floor in which the support failed, therefore, the apical prolapse will correspond to a prolapse of the uterus of top of the vagina. A cystocele is a prolapse of the anterior part of the pelvic floor, and a rectocele represents a prolapse of the posterior area [9].

Fascia

The pelvic fascia wraps the pelvic walls and pelvic viscera, as well as, forms sheets of tissue around blood vessels and nerves. It is divided in the parietal pelvic fascia, which is related with the pelvic muscles, and the visceral pelvic fascia which enfolds the pelvic visceral and their vessels and nerves.

Ligaments result from the condensation of the fascia, such as, the pubocervical ligament, transverse cervical or cardinal ligament and uterosacral ligament, extending from the cervix to the anterior, lateral and posterior pelvic walls, respectively. The support and stability of the uterus and pelvic cavity is thought to be ensured by these ligaments, as well as, the muscular component of the pelvic floor. The cardinal ligaments are considered important since they extend laterally from the sides of the cervix and vaginal vault, attaching it to the pelvic wall. The uterosacral ligaments maintain vaginal length and keep the vaginal axis horizontal in standing position.

Peritoneum

The pelvic peritoneum is a continuity of the abdominal peritoneum originating at the pelvic inlet. It covers the pelvic viscera and forms ligaments between the viscera and pelvic walls. The broad ligaments is a sheet-like fold of peritoneum running from the lateral pelvic wall to the uterus, enclosing the uterine tube its top margin and suspending the ovary from its posterior aspect. The ligament of the ovary and round ligaments of the uterus are incorporated in the broad ligament. The broad ligament is divided in three parts:

- The mesometrium, which is the largest part and extends from the pelvic floor to the ovarian ligament and uterine body. Is encloses the ovarian vessels and nerves. It also englobes the proximal part of the round ligament of the uterus, as well as smooth muscle and loose connective tissue;
- The mesosalpinx is the top part of the broad ligaments and suspends the uterine tube in the pelvic cavity. Posteroinferioly it is connected to the mesovarium.
- The mesovarium is the posterior extension of the broad ligament, which attaches to the ovary. The ovary ligaments if a fibromuscular strip of tissue, located medially in the margin of the mesovarium to the uterus, then continues laterally as the round ligament.

The round ligaments bands of tissue with approximately 10 cm long that pass over the pelvic inlet. This ligament gradually turns from a purely fibrous tissue to a fibrous tissue with smooth muscle cells, near the uterus. The round ligament also encloses blood vessels and nerves.

Endopelvic Fascia (EF)

The EF is a fibromuscular layer which attaches the cervix and vagina to the lateral pelvic wall, it includes part of the PD and a visceral component. The latter is situated below the peritoneum and provides support for the bladder, vagina, uterus and rectum. As for the visceral component, this has different types of tissues in different parts of the fascia [3].

Pelvic Ligaments (PL)

The PL are contractile structures with a main role in maintaining the support of the pelvic viscera, and these result from condensation of the EF. The difference in tissues is not always clear, but the ligaments referred here are the primary ligaments in terms of support of the pelvic organs [17].

Tabela 2. 1– PL involved in support of the pelvic organs grouped in terms of support location [17]

Support level	Ligament	Supported structures
1	Uterosacral (USL) Cardinal (CL)	Cervix
2	Pubovesical (PVL)	Bladder
3	Puborectalis (PRL) Anococcygeal (ACL)	Urethra Anus

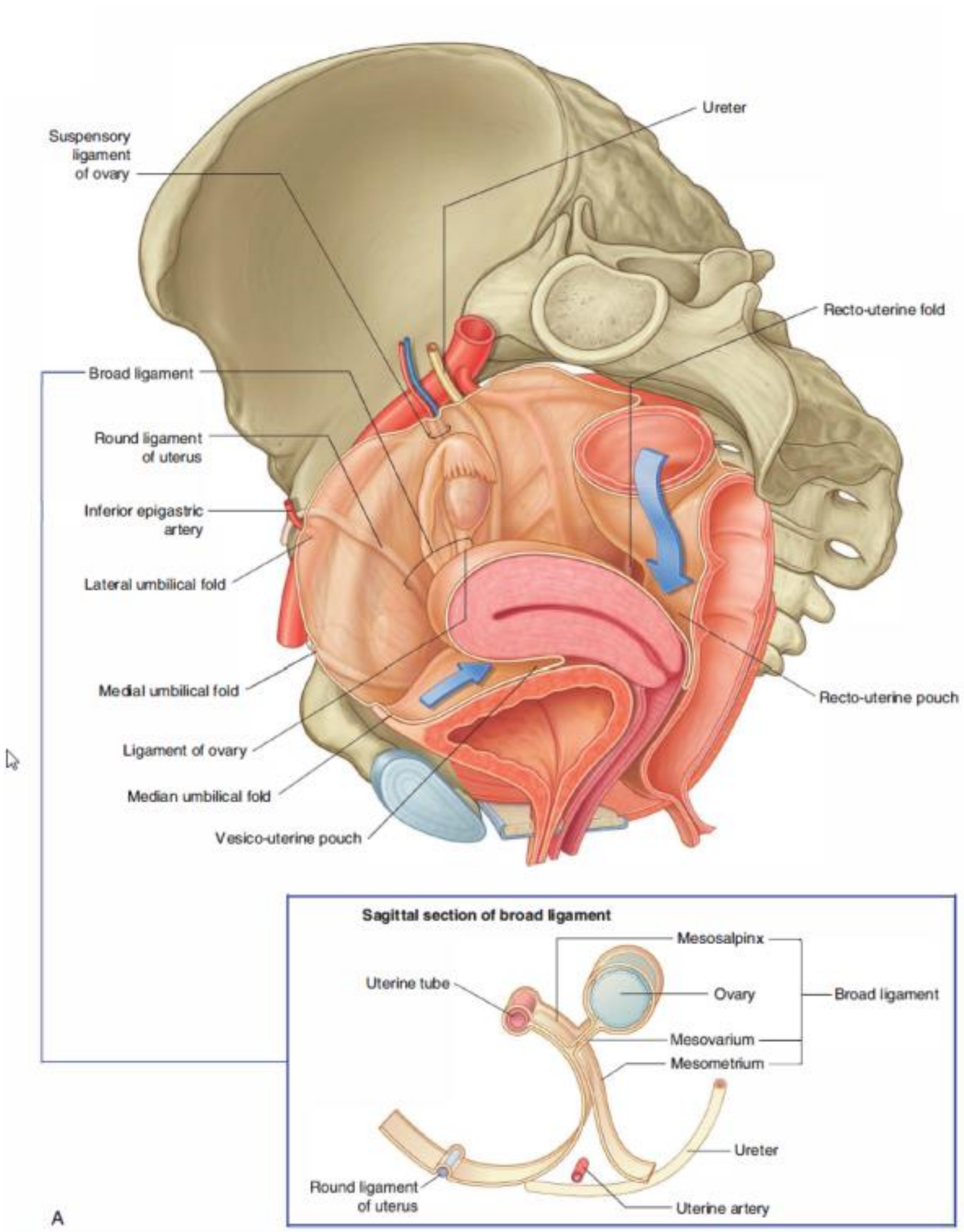


Figure 2. 7 - Female peritoneum adapted from Drake et al.[10]

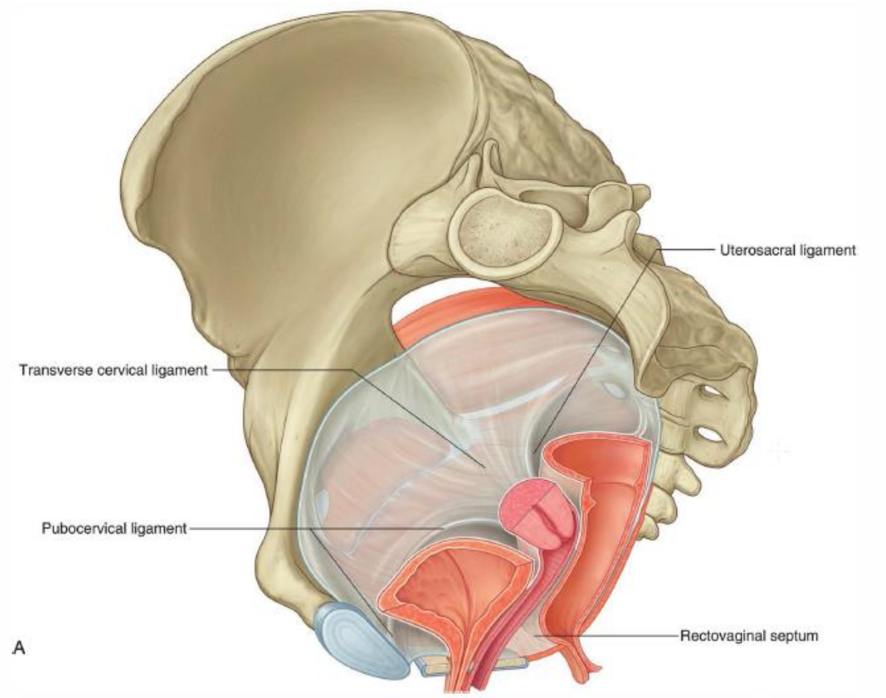


Figure 2. 8 – Pelvic fascia adapted from Drake et al. [10]

2.5. Organs of the pelvic cavity

Urethra and Bladder

The urethra consists of a tubular organ connecting the bladder to the urinary meatus, taking different paths in different genders. This organ is formed by an inner layer of smooth muscle and an outer layer of striated muscle, the latter being the one responsible for maintaining continence [9, 11].

The bladder is located posterior to the pubic symphysis, acting as a urine reservoir, until physiological capacity is reached, expelling the urine through the urethra. The retropubic space lies between the bladder and the pubic bone, which contains supportive ligaments near the bladders base. The bladder has three layers of tissue: an inner mucous membrane, a smooth muscle layer and an outer layer, covered by peritoneum in the upper part. Anatomically the bladder can be divided into cupula, body and base. The cupula corresponds to the top part and is covered by the peritoneum. The second part corresponds to the largest part of the organ, connecting the bladder to the kidneys through the urethers. The base corresponds to the bottom of the bladder connecting it to the urethra. This organ, with distention, is raised over the pubic bone, with its base maintaining position [9, 18].

Vagina and Uterus

The vagina is a tubular tract, connecting the vulvar opening to the uterus, even though the vaginal tract itself ends at the cervix. It has an inner mucous membrane, containing the glands responsible for the secretions characteristically of the organ, and an outer muscular layer[9, 15, 18].

The uterus is a multilayer organ in the shape of an inverted pear, located above the bladder and anteriorly to the rectum. It is connected to the cervix and the fallopian tubes. The uterine walls have three layers: the endometrium, the myometrium and the perimetrium.

The perimetrium is the outer layer and consists of visceral tissue, as for the myometrium, it is a muscular layer. The endometrium, which is the inner layer is a mucous layer. The uterus is 85% muscular tissue and 15% connective tissue [14, 15, 18].

<u>Rectum</u>

The rectum is the final portion of the large intestine, following the shape of the sacrum. Comparing to the rest of the intestine, this last part is muscularly reinforced, extending from the end of the sigmoid colon to the anus [15, 18].

2.5.1. Support of the pelvic organs

<u>Uterus and Vagina</u>

The uterus and vagina support can be presented in three levels, from top to bottom, the perimetrium on top, the CL and USL in the middle and the LA on the bottom. The perimetrium is also the outer layer of the uterus, and will by the interacting with the support structures. The CL attaches the side of the cervix to the ischial spine. The USL attaches the posterior cervix to the sacrum. These two ligaments are completed by the PVL and rectovaginal, which attaches the cervix to the lateral walls of the pelvic floor. The same ligaments also form the PVL, which is responsible for supporting the vagina anteriorly, attaching it to the tendinous arch of pelvic fascia (TAPF), and rectovaginal fascia (RVF), which supports the vagina posteriorly, attaching it to the PB. On a lower lever the vagina is laterally supported by the LA [3, 18].

<u>Bladder and Urethra</u>

The urethra is supported by the pubourethral ligament (PUL) and by the external urethral ligament (EUL), both originating from the pubis symphysis supporting the urethra medially (PUL) and anchoring the external urethral meatus (EUM). The PUL also extends into the PCM [3].

The bladder’s anterior wall support is provided by the PVL, which is located between the detrusor muscle (DM) and the TAPF [3].

<u>Rectum and Anus</u>

The rectum is mainly surrounded by fat tissue and its support is granted by the adjacent connective tissue and anorectal fascia. The lateral support is given by the condensation of the EF. The RVF and the presacral fascia (Waldeyer’s fascia) ensures anterior and posterior support respectively [3].

The anus is supported anteriorly by the PB and associated structures of the anovaginal septum. The pubovisceral muscle and the transverse perineal muscle gives lateral support. The anococcygeus ligament provides support on the posterior face, attaching it to the coccyx [3].

2.6. Dynamics of the pelvic floor

Biomechanics is the study of biological structures and systems, by means of classic mechanics. The study of biomechanics of the female pelvic floor as allowed a better understanding of its function of its dysfunctions.

2.6.1. Urethra opening and closing

The action of opening and closing by the urethra is due to the contraction and relaxation of the PCM. The contraction of the pubococcygeus and the PRL suspends the urethra, allowing the LA and the deep transverse perineal muscles to pull the proximal urethral, maintaining it closed. On the other hand, when the PCM is relaxed, the action of the LA and deep transverse muscle is maintained and the urethral orifice is opened [8].

The urethra has three normal states, each resulting of a different combination of muscle forces [8]:

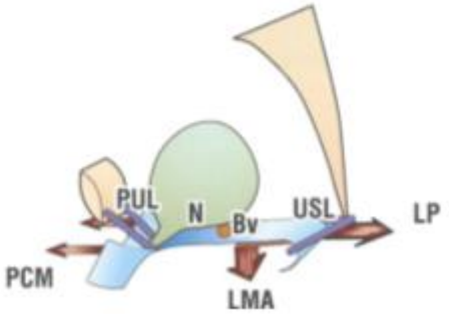
- **Resting closed**: the muscle contraction and elasticity of the vagina maintain the urethra closed. The distal part of the vagina is subjected to a force applied by the contraction of the anterior PCM, proximal LA and deep transverse muscles;
- **Closure during effort**: the vagina and urethra are pulled by the CM, acting over the urethral closure mechanism, and the deep transverse and LA muscles act over the proximal urethra permitting the closure of the bladder neck mechanism;
- **Opening during micturition**: the PCM is relaxed, allowing the force from the LA and deep transverse muscle to open the bladder neck and urethra.

2.6.2. Anorectal opening and closure

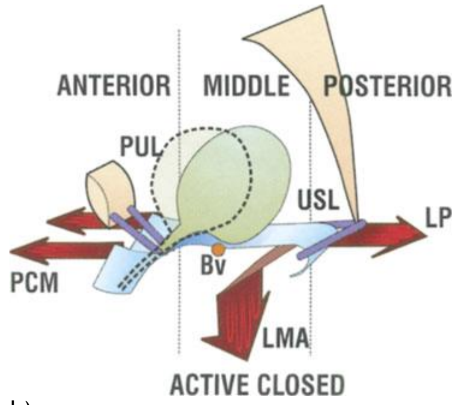
The opening and closing action of the anorectal opening is directed by four forces, resulting of the action of the PRM, PRL and USL and the LA muscle. During defecation the anorectal opening will allow the evacuation of the feces, three structures are involved: LA, the deep transverse muscle and the PCM. During the closure of the anorectal opening the LA pushes anteriorly the rectum to the anus. After being extended, the deep transverse muscle will act over other structures located below it, creating the angle of the anorectal area and maintaining the opening closed. During expulsion of the feces the PRM is relaxed and the three other structures are contracted [8].

2.6.3. Pelvic floor during the Vassalva maneuver

The vassalva maneuver consists of an attempt to exhalation while maintaining closed airways, which is usually achieved by closing the mouth and pinching the nose. This will cause an increase in internal pressure and from the perspective of the pelvic floor, it can be used to perform several diagnosis while the muscles are relaxed. A greater intra-abdominal pressure causes a descend in the level of the pelvic floor, expansion of the urogenital hiatus and anal canal, distention of the anococcygeus ligaments and a posterior displacement of the pelvic organs. Anteriorly the bladder and urethra rotate around the pubic symphysis. Posteriorly to the bladder, the vagina is moved posteriorly and the uterus will occupy a vertical position above the vagina. In the posterior area of the pelvic floor, the rectum may cause a perturbation of the posterior wall of the vagina.



a)



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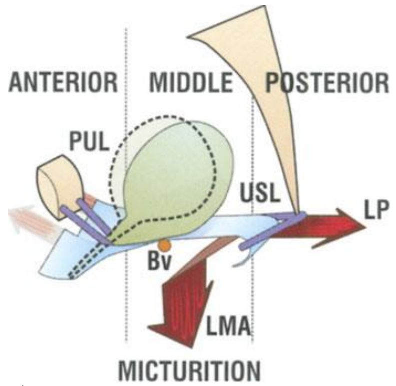


PUL - pubourethral ligament
USL – uterosacral ligament
PCM – pubococcygeus muscle
LMA – deep transverse muscle
Bv- bladder's fixation point to the vagina

c)

Figure 2. 9 - Representation of three state of urethral configuration: a) resting closed; b) opened during effort; c) opened during micturition; The dote line represent the final position of the bladder in each situation [8].

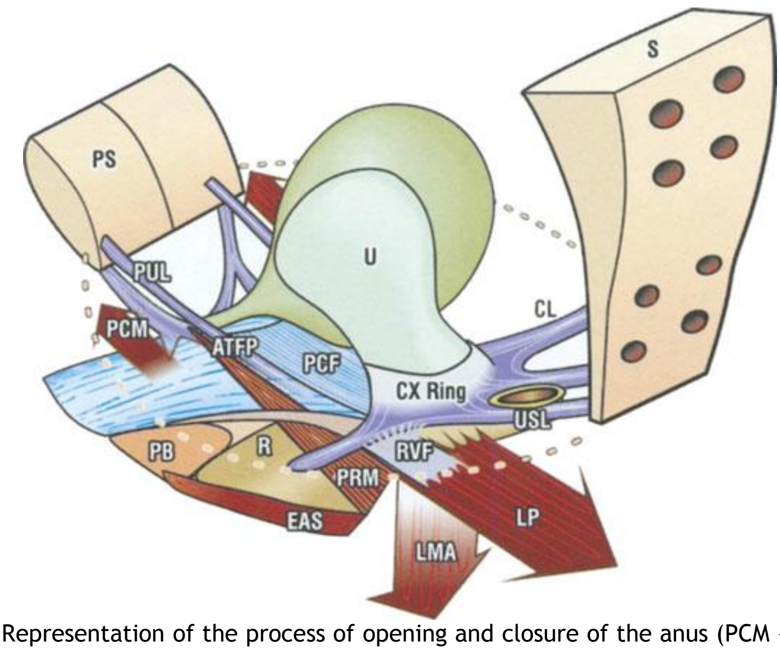


Figure 2. 10 - Representation of the process of opening and closure of the anus (PCM – pubococcygeus muscle; LP – levator ani muscle; LMA – deep transverse muscle; PUL - pubourethral ligament; RVF – rectovaginal fascia; EAS – external anal sphincter; PRM – puborectalis muscle) [8].

During defecation, if the PRM is not relaxed during the maneuver, there can be an obstruction. In case of genital prolapse the vagina may descend to the outside of the body, and can be followed by the uterus [19].

2.7. Morphology and mechanical properties of pelvic tissues

Here a list of scientific evidence is presented, regarding the morphology and mechanical properties of pelvic tissues. This also gives a general idea on the evolution of the study of these tissues.

In 1987 the biochemical alterations of the connective tissues from women with SUI were investigated. The study concluded that women with SUI had 40% less collagen in the skin and round ligaments of the uterus, suggesting a deterioration of the connective tissues in women affected by the dysfunction [20].

A study in 1994, conducted on fibroblast, concluded that women with SUI have an altered connective tissue metabolism with a difference of 31% in collagen production, compared to continent women. This may be related to the dysfunction on the pelvic floor [21].

Also in 1994 the amount of collagen type III was studied by Bergman et al in pelvic supportive tissues of women with SUI. The results suggested that women with SUI have an altered collagen concentration in skin, USL and round ligaments [22].

In 2000, Fitzgerald et al found alterations in urethral collagen morphology in women with SUI, with no difference on diameter of the collagen fibrils [23].

In 2002 Iida et al. discussed the influence of aging on the mechanical properties of lumbar supraspinous and interspinous ligaments. The results showed a decrease in ligament strength [24].

The interspinous ligaments were also studied by Barros et al. and the results showed loss of elasticity, caused by the alteration of the composition or configuration of collagen and elastin [25].

In 2003 the results obtained by Goepel et al. [26] show a decrease of collagen type I, III and VI as well as absent or fragmentation of vitronectin in continent and incontinent postmenopausal women with genital prolapse.

Also in 2003, Cosson et al studied the strength of PL. The samples were obtained from cadavers and tested under different storage conditions. The values obtained varied from 20 N to 200N, being the strongest the pre-vertebral ligament, while the iliopectineal ligament and the sacrospinous showed results around 20-31 N [27].

In 2006, included in the thesis of Janda, a study was performed in order to identify morphological parameters of several structures from pelvic diaphragm. The samples were obtained from the cadaver of a 72 year old woman and focused on obtaining information on the size and orientation of the muscular fibers [28].

In 2007 Rubod et al [29] established an experimental protocol to characterize the mechanical properties of vaginal tissue, ensuring that this would provide good test repeatability. They tested the influence of freezing, hygrometry, sample location and orientation, temperature conditions and deformation rates.

In 2010 Peña et al [30] investigates the viscoelastic mechanical properties of vaginal tissue. The study aimed to present a model of the viscoelastic properties, even though the tissue used was anisotropic, uniaxial tensile tests were conducted.

A review published by Tinelli et al. [31] focused on the modifications and risk factors of the postmenopausal woman. Even though the criteria used for the classification of prolapse are vague and in some cases unclear, aging tends to lead to an overall decrease in mechanical strength. This leaves woman more exposed to dysfunctions such as pelvic prolapse.

In 2011, Martins et al assessed the mechanical behaviour of the female bladder, by performing uniaxial tests on tissue samples. The bladder tissue showed a mean stiffness of 1.9 MPa and a mean maximum tension of 0.9MPa. The results also showed that tissue from women younger than 50 years shows higher stiffness than those from older women, however, maximum bladder stress could not be related to age [32].

In 2012 Martins et al. [33] investigated the tensile biomechanical properties of round ligaments and USL. They obtained tissue from 15 female cadavers, with no PFD, and performed uniaxial tests of the sampled tissues. The USL showed higher stiffness and maximum stress compared to the round ligaments. This study did not take under consideration age, body mass index and menopausal status, but demonstrate lower USL stiffness on nulliparous women.

In 2012 Rubod et al [34], with the information obtained in 2007, demonstrated a nonlinear relationship between stress and strain and a visco-hyperelastic behaviour in pelvic tissue. They classified organs and ligaments in terms of rigidity and extendibility, and showed different behavior in the different organs and ligaments in larger strain levels.

In 2013 Martins et al [35] compared the biomechanical properties of vaginal tissue in women with and without POP. The tissues were characterized in terms of stiffness and maximum stress, and these parameters were obtained through uniaxial tension tests. The results obtained showed different properties in the two cases, being the tissues from non-POP women the ones presenting lower values of stiffness and maximum tension.

In 2014 Chantereau et al [36] conducted a study to test the mechanical properties of soft tissue in young women and the impact of aging. Uniaxial tension tests were performed on pelvic soft tissues from organs and ligaments. The results obtained revealed that aging and trauma to the tissues modify the mechanical properties of the pelvic tissues. As a woman ages, the tissues present in the pelvic floor will acquire different behaviour. The same results were also suggested in older studies.

Chapter 3.

Pelvic floor disorders

As time passes and age naturally takes its toll or some trauma to the pelvic tissues is suffered, some problems may arise, due to mechanical or biochemical factors. These could be menopause, hormonal therapies, childbearing and other issues in which the pelvic floor will suffer alterations. Dysfunctions of the pelvic floor are mainly associated with age and/or childbirth. In earlier stages of life the lack of tonus from one muscle is easily compensated with the surrounding muscles but as a woman ages, the surrounding muscles may also lose tonus, and the whole system can collapse since the muscles which were compensating are no longer capable. Natural childbirth implies trauma to the tissues, which can lead to permanent damage. The three pelvic floor dysfunctions are: prolapse, urinary incontinence and faecal incontinence [3].

3.1. Pelvic Organ Prolapse (POP)

POP is a condition in which the organs protrude through the vagina which happens when the support structures fail. The prolapse can be characterized by the area of the pelvic floor affected and by the degree of protuberance [3, 5].

3.1.1. Physiopathological characterization

The prolapse is caused by the rupture or failure of the support structures. In a normal situation, a non-pathological scenario, the PCM and iliococcygeys muscles and puborectalis are contracted, in a passive situation, ensuring the closing of the urogenital hiatus, allowing a stable support plane for the pelvic organs. An alteration of the muscular tonus of the LA will jeopardize the stability of the pelvic viscera [4, 5].

As referred in tables 3.1 and 3.2, the prolapse can occur on the anterior, posterior or apical. An anterior prolapse is characterized by the descent of urethra and bladder to the anterior wall of the vagina, the posterior prolapse affects the anus and associated structures, which will descend to the posterior wall of the vagina. The anterior prolapse is related with the cardinal ligament (CL) and uterosacral ligament (USL) as well as the pelvic diaphragm

(PD). This one being related with uterovaginal prolapse, due to its relation with the vagina and uterus [3, 4].

Table 3.1 – Classification of prolapse based on structures that descent [5]

Terminology	Description of the prolapse
Urethrocele (anterior)	Prolapse of the inferoanterior vaginal wall involving the urethra only
Cystocele (anterior)	Prolapse of the superoanterior vaginal wall involving the bladder, usually associated with urethrocele, therefore the term cystourethrocele can be used
Uterovaginal (apical)	Prolapse of the uterus, cervix and top part of the vagina
Enterocele (posterior)	Prolapse of the superoposterior wall of the vagina
Rectocele (posterior)	Prolapse of the inferoposterior wall of the vagina involving the appperance of a protuberance created by the rectum

Table 3. 2- Classification of prolapse based on the descent level[5]

Degree	Description
I	Halfway to hymen
II	To the hymen
III	Halfway past hymen
IV	Maximum descent

The apical prolapse is also related to the insertion of the vesicovaginal fascia of the perivesical ring, as well as, with the lateral stability of the tendineus arch. The perivesical ring, which is formed by the SUL, CL and EF, is responsible for the junction of the cervix to the uterus. The failure of these structures can result in lack of support of the posterior vaginal wall, PM and perineal body, leading to a posterior prolapse [3, 4, 28].

3.2. Urinary incontinence

In a non-pathological scenario, the storing of urine is controlled by central and peripheral mechanisms. The central mechanisms are the innervation of the urinary tract, as for the peripheral mechanisms, these correspond to the organs tissue and the muscles associated with these, as well as, the muscles of the pelvic floor which are associated to the same organs. The continence of urine is ensured by the combination of these two [3, 5].

This dysfunction of the urinary system is defined by the involuntary loss of urine, being divided into three types: stress urinary incontinence (SUI), urge urinary incontinence (UUI) and mixed urinary incontinence (MUI) [5].

3.2.1. Physiopathological characterization

Stress incontinence is characterized by the loss of urine related with the rise of intra-abdominal pressure, this can occur due to physical effort or just by coughing. Urge incontinence is related to an urgent need to urinate, leading to an involuntary leak. The mixed incontinence is cause by a combination of both [5].

Urinary incontinence occurs when there is a failure at some level over the control systems that the body possesses and there are some hypothesis for the occurrence of incontinence in the urinary tract [8]:

Alterations of the uterocervical axis

This consists of the displacements of the urethra and uterocervical junction to the pubic symphysis, compromising the transition of pressure from the bladder to the urethra, resulting in a deficient closure of the sphincter [5].

Sphincter dysfunction

In a stress situation the relaxation of the proximal urethra can occur, or the opening of the bladders base, resulting in the loss of urine due to the failure of the sphincter mechanism [5].

Hammock theory

Knowing that the support of the urethra is ensured by the EF and anterior wall of the vagina, and that these structures are supported by the tendineus arch of the pelvic fascia and the LA, a failure in one of these structures can lead to urinary incontinence. In this case, the pressure transmission will not occur correctly from the bladder's base to the proximal urethra, resulting from the lack of support from the referred structures [5].

Integral theory

In this case, the incontinence will be caused by a lack of elasticity of the tissues associated with the anterior vaginal wall, resulting in the activation of the receptors present in the bladder base and proximal urethra, resulting in involuntary urination [8].

3.3. Faecal Incontinence

Faecal Incontinence is the lack of control over the expulsion of gases and/or faeces. It can be classified as Passive, Urge and Mixed Faecal Incontinence.

Passive Faecal incontinence occurs when there is a dysfunction of the internal anal sphincter. On the other hand an Urge Incontinence is characterized by the failure of the external sphincter. As for the Mixed incontinence, it has symptoms of both [3, 4].

Natural childbirth is a major risk factor for faecal incontinence, mainly associated with PFI, since this puts the tissues under a lot of stress, and in some cases an episiotomy is required, if performed incorrectly the damage to external anal sphincter may be permanent [3].

3.3.1. Physiopathological characterization.

PFD can have several causes, the most common being sphincter anomalies and neuropathy. Around 80% to 90% of cases have some sort of sphincter lesion, and 20% to 36% show problems on a neuronal level. Other factors can contribute to faecal incontinence, such as the compliance of the tissues and inappropriate sensation, which can be found in less than 5% of the cases. Some other factors can lead to more severe problems, like, alteration in the consistency of the faeces, abnormal retention or even idiopathic disease [3]. In the case of sphincter lesions, as referred previously, one important cause is episiotomy, which can lead to severe complications on the external anal sphincter.

As referred previously, the main cause for faecal incontinence is sphincter related problems and this is usually revealed in later stages of life, since in younger women the surrounding muscles can compensate for the lack of some of its neighbour muscles. The PRM responsible for maintaining the position of the rectum and anus, which by remaining contracted, will ensure that there is no loss of faeces [37].

The rectum ensures the faecal continence through its compliance and the sensation of rectal filling. These two are intimately related, since the sensation will depend on the compliance of the tissues [37].

3.4. Epidemiology

The epidemiology of the pelvic dysfunctions allows the study of the incidence and prevalence of these problems in a population. The results are not always accurate since studies may use different criteria and the fact that symptoms associated with the dysfunctions are sometimes non-specific. Urinary incontinence is defined by the International Continence Society (ICS) as “the complain of any involuntary leakage”, Faecal Incontinence has different definitions and will depend of the population surveyed and prolapse has nonspecific symptoms, as well as the fact that an accurate diagnosis depends on physical exam. These criteria have a wide spectrum of acceptance, and will ultimately depend of the physician that performs the examination, and the examined patient [38].

Urinary incontinence

- Prevalence of urinary incontinence in general population of women, in most studies, ranges from 25 to 45%;
- Older women have a higher probability to develop mixed and urge incontinence;
- Middle-aged women are more likely to develop stress incontinence;
- The studies considered showed that half the incontinent women present signs of stress incontinence, followed by a lesser number of mixed incontinence, being the least fraction correspondent to urge incontinence;
- Through the age range, the results showed an increase in prevalence of mixed incontinence, also showing a decrease in stress incontinence from the 40-49 through 60-69 age group;
- During pregnancy studies show results ranging from 33% to 64% for all incontinence type;
- The probability to develop urinary incontinence increases as the pregnancy unwinds;

- Black women, when compared to white women, show less tendency to urinary incontinence.

Faecal Incontinence

- The prevalence for Faecal incontinence in adults is reported to be between 11% and 15%, even though the reports considered do not specify the degree of disability;
- Obese women have a higher risk do develop faecal incontinence;
- Obstetric trauma, such as lesion of the pudendal nerve or sphincter (episiotomy), is a primary risk factor;
- Irritable bowel syndrome is also a major risk factor, which is also more prevalent in women than men.

Pelvic Organ Prolapse

- Stage I and II prolapse occurs in up to 48% of women;
- Stage III or IV prolapse is found in around 2% of cases;
- Anterior vaginal prolapse, or Cystocele, posterior vaginal prolapse, or Rectocele, and Uterine prolapse is found in 9.3, 5.7 and 1.5 per 100 cases annually ;
- In women with any kind of prolapse, progression occurred in 10.7%, 14.8% and 2.0% cases of anterior, posterior and uterine prolapse per year;
- Regression of anterior vaginal prolapse from grade I to No-POP occurs is 23.5% of cases per year, and from grades II and III to No-POP in 9.3% of cases annually;
- Posterior prolapse is regressed in 25.3% of cases and in 48% of cases for uterine prolapse annually.

3.5. Diagnosis

To ensure a successful treatment first the diagnosis must be correct, thus in this chapter a revision over the diagnosis options will be given [3].

3.5.1. Physical examinations

A first stage in the diagnosis includes a series of physical examinations to identify possible markers for incontinence or prolapse, these can be of neural origin [3, 17]. The exams will allow the evaluation of the neural capacity, as well as, the state of the tissues, and the exams should indicate [17]:

- Check for atrophy of the Vulva and Vagina, which can be an indicator for low oestrogen;
- Manual examination to access and locate the origin or existence of pain;
- Rectal exam to aces the integrity of the LA;
- Check for involuntary loss of urine or faeces;
- Classification of the type of prolapse, to know the extension of the damage;
- Urethral examination by palpation of the vaginal anterior wall and check for urethral atrophy or presence of hard structures present in the organ.

3.5.2. Urodynamic tests

Urodynamic testing allows assessment over the functionality of the bladder and urethra. There are several types of tests including: urofloximetry, cistometry, pressure/flow assessment, electromyography of the urethra sphincter, video-urodynamics, Vassalva maneuver tests and profilometry[4, 28].

For instance, to assess the discharge pressure with the Vassalva maneuver, the bladder must be filled with 310mL during the test, and the patient is asked to perform the Vassalva maneuver while the intra-abdominal pressure is measured using a rectal catheter. The pressure corresponding to the moment of discharge is related to incontinence [4].

- If it is superior to 100cmH2O, the incontinence is a result of the failure of the bladders base support;
- If it is inferior to 60cmH2O, there should be a dysfunction of the internal urethral sphincter;
- These test are performed to assess information on the;
- Hyperactivity of the DM and its function during urination;
- Capacity of the urethra and bladder to retain urine;
- Obstruction on the base of the bladder.

3.5.3. Imagiology

Imagiology techniques allow the study of the human body without the need directly access the structures. In the case of the pelvic cavity, it allows the study of the anatomy of the area without the need to cut the patient [3].

The examination of the pelvic cavity relies majorly on two technics: magnetic resonance imaging (MRI) and medical ultrasonography techniques (MUS), as for conventional X-rays, these are not very useful when studying soft tissues [3].

The evolution of MRI now allows the study of moving structures (dynamic MRI), allowing a deeper study of the muscles, ligaments, organs and other structures of the pelvic floor. The examination usually has three steps: the contraction of the anal muscles and elevation of the pelvic floor to assess the contraction of the PRL; the process of emptying the bladder and the intestine is simulated to check for rectal and urethral dysfunctions and the existence of prolapse is checked with the Vassalva maneuver [3].

Medical ultrasonography (MUS) is also an alternative to examine the pelvic floor. This technic can be used to assess the condition of the urethra, bladder, vagina (including cervix and low uterus) and rectum by placing the transductor over the pubic symphysis. To assess the rectal function an endosonography can be used to perform a diagnosis on the anal sphincters [3, 19].

3.6. Treatments

As in other medical areas, the treatments available for the dysfunction of the pelvic floor can be conservative and surgical. Each has its advantages; therefore, an evaluation must be performed to determine the most suitable treatment.

3.6.1. Conservative treatments

These treatments are used in every case as an attempt to prevent surgery. Talking of conservative treatments include several treatments to be used without the requirement of surgical intervention, as a way to avoid surgical treatments[5, 37]. These are:

- Education of the patient for a better understanding of the pathologies;
- Changes in life-style of the patients, including diet and other habits;
- Pharmacological treatments for incontinence, acting on the nervous system over the contraction of the muscle involved;
- In case of prolapse, the use of a pessary, which is a device placed inside the vagina that, in this case, will provide structural support;
- Muscle training of the pelvic floor, involving the voluntary contraction by the elevation and opening/closing action of the anal, vaginal and urethral orifice;

3.6.2. Surgical treatments

When non-invasive treatments fail, surgical treatments are used as an alternative. Is estimated the 11% of women go through pelvic surgery once in their life [6]. The identification of the structures present in the pelvic floor is not an easy task and in 50% of cases a second surgery is needed, due to incorrect identification. Classic technics are usually more invasive, and surgery to the pelvic floor shows a rate of 7% to 36% for post-surgery complications. In order to minimize this rate, less invasive alternatives were developed and in here an example for each main dysfunction is given [6, 39].

Transvaginal Mesh

Surgical mesh were originally designed for hernia surgery, but later on started to be used in the treatment of pelvic dysfunctions. Vaginal mesh procedures were introduced in 2003 and approved in 2004 in the USA, and are mainly used in cases of Pelvic Organ Prolapse, being also an alternative in cases of urinary incontinence. These are used as a potentially durable, less invasive with fewer complications compared to other traditional procedures [39].

The mesh itself will provide a permanent support to the pelvic viscera and as in all prosthesis the material will be encapsulated in collagen due to the response of the immunity system. The results referred by Sanses et al. [39] are based in cases where the mesh was macroporous polypropylene prosthesis and showed 78% to 100% in long term cure rate for apical prolapses.

Slings

Sling surgery is mainly used for stress urinary incontinence, and consists of introducing a sling around the urethra, allowing the elevation of the urethra to its anatomical correct position [40, 41].

Artificial Bowel Sphincter

This procedure is used for faecal incontinence, in cases where the cause is dysfunction of the anal sphincter, generating pressure on the sphincter itself closing it artificially. The device is an artificial cuff implanted around the anal canal, a control pump and a pressure regulating balloon positioned anterior to the bladder [42]. These devices are reported to be associated with complications, such as, infection in 20% or more of cases and 50% required explanation due to dysfunction and erosion of the device [42].

Wong, et al. [43] reported that 68.8% had a successful implantation of the device. The other 41.2% experienced device-associated infections in early or later stages.

Chapter 4.

Pelvic Ligaments

4.1. Histology

Ligaments are primarily formed of type I collagen fibres, which are dense and highly oriented tissues. The direction taken by the fibres is a translation of the loads sustained by the ligament. This tissue is usually constituted of 20% of cells, 56% of water and 24% extracellular matrix[44, 45]. This matrix is composed primarily of collagen, ground substance and small portion of elastin. The collagen fibres are the main responsible for the load-bearing capabilities of ligaments and constitute over 75% of the mixture. Elastin is a protein which shows behaviour similar to a rubber. As for the ground substance, it is a gel-like substance formed by proteoglycans (PGs) and other glycoproteins embedding the fibres [44-46].

Unlike other ligaments, the pelvic ligaments result from the thickening of fascial tissue and enwrap blood and lymphatic vessels, as well as nerves and fat tissue [1]. The extra-cellular matrix is formed of collagen, elastin and other proteoglycans. This matrix is maintained by fibroblasts, modulating the synthesis and breakdown and renewal of the fibres forming the matrix [47]. Apart from the basic constitution of the ground substance (non-collagenous glycoproteins, proteoglycans and hyaluronan), in the case of pelvic ligaments, this also includes a significant amount of smooth muscle cells [48].

The collagen fibres, in healthy and young ligaments, are mostly formed by type I collagen. These molecules organize in collagen fibrils, forming long filamentous chains which aggregate forming the collagen fibres [7]. These characteristics are the main responsible for the biomechanical properties of the tissue. The ratio of collagen and elastin is closely regulated by the fibroblasts, and will directly influence the response of the tissue [48]. Type I collagen is mostly present in flexible tissues with great resistance to tension, therefore “strong” tissues. On the other hand, type III collagen is present in tissues which require more flexibility and distension, usually subjected to periodic stress. Type III has more significant expression in vaginal tissue, for example [47, 48]. With aging type I collagen percentage decreases, being substituted by type III, resulting in a slacker tissue.

When relaxed a collagen fibre presents the form of an S, acting as a spring, damping the forces which would otherwise be transferred directly to the adjacent tissues. When totally stretched, the damping effect will be practically inexistent. The tissues ability to distend when submitted to a force will depend of the configuration and orientation of the fibres, also characterizing its anisotropic response [8]. Due to these characteristics, the collagen fibres are responsible for the response of the ligament to traction stimulus. On the other hand, the ground substance with the aid of the fibrous structure of the ligaments, will respond in a compressive scenario [44].

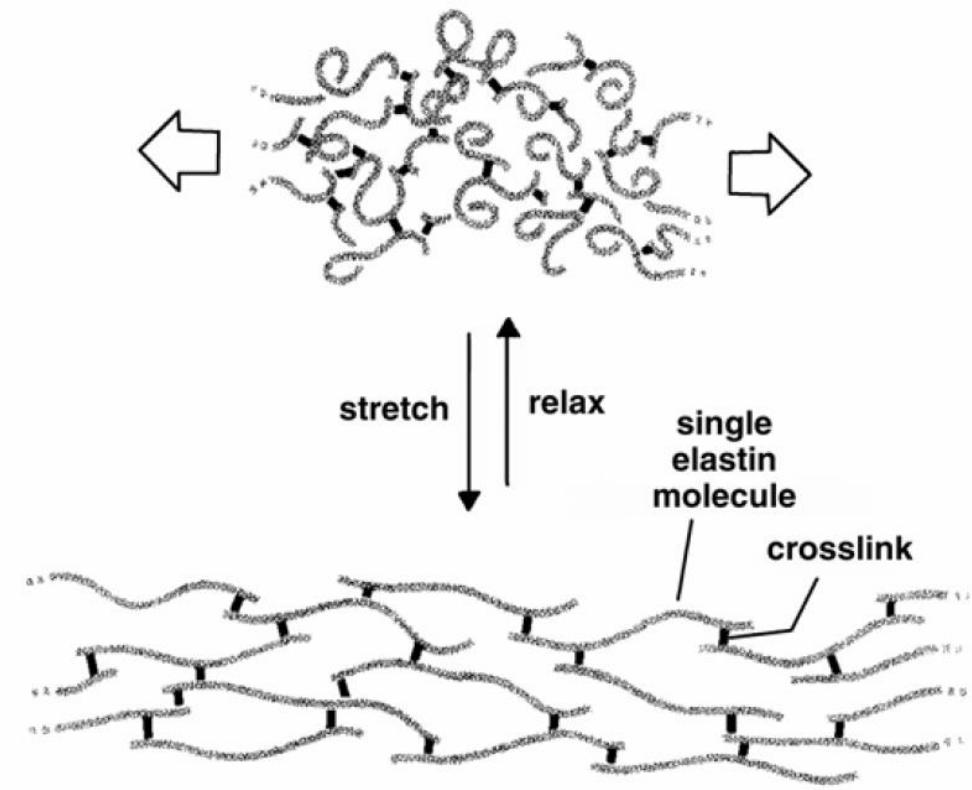


Figure 4. 1 - Difference between elastic fibres while relaxed (top) and stretched (bottom),

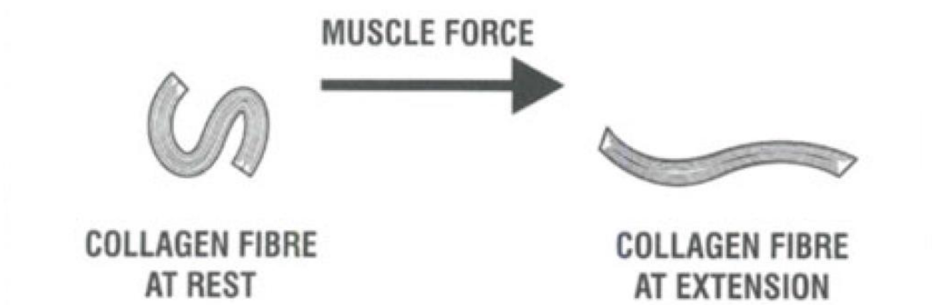


Figure 4. 2-Difference between collagen fibres while relaxed (left) and stretched (right); The image on the left present the S shaped fiber.

Increased levels of collagen type III and V decrease the mechanical resistance of the ligaments, since these types of fibres are known to be smaller. The ratio of collagen type I and III is also related to the mechanical response. With higher percentage of type I collagen the tissue will be more resistant.

Elastin content is also important for the mechanical characterization of the tissue. This is an rubber-like polymer, which allows the tissue to recover its original configuration after it's stretched, with no external energy required. This is extremely important in scenarios where the tissue is submitted to extreme stretches, such as childbirth. During the gestation period, and even more during delivery, the tissues of the area are taken to their extreme, and should recover its original configuration some days after [48].

The increase of collagen fibres and decrease in fibroblast numbers is related to pelvic relaxation. A healthy tissue is an active tissue, meaning that there is constant (cellular) activity in the tissue with constant remodelling and repair of the matrix. In the same way as other types of tissues are able to regenerate (such has bones), when a ligament suffers damage it can be able to self-repair. A scar will appear in a repaired tissue, which can appear in the form of a fibrosis. In this area the tissue will lose elasticity and become more fragile.

An increase in collagen fibres does not necessarily represent a healthier tissue. In cases of genital prolapse the increase of collagen fibres can be the cause of the problem, since the type of collagen increased is the type III. This is a key point in the diagnosis of pelvic disorders [49]. It can also be related to aging.

4.2. Aging

Aging is transversal, universal and intrinsic process to all life forms. In the human body it has several effects through different means. This process is built-in in our genetic design. On a higher scale the soft tissues and organs change, affecting their performance, health and strength. On a cellular level, the cells overall capacity to multiply themselves and also replace and repair the matrix will be affected, consequently affecting the tissue [50].

Ligaments are known to express viscoelastic properties, but this behaviour changes as the tissue matures. The mechanical behaviour is determined by the properties and quantities of each component and their interactions. Also, ligaments have time- and history-dependent viscoelastic properties, meaning that their properties evolve as a person ages, but also retain changes caused by some kind of trauma (or lack of it).

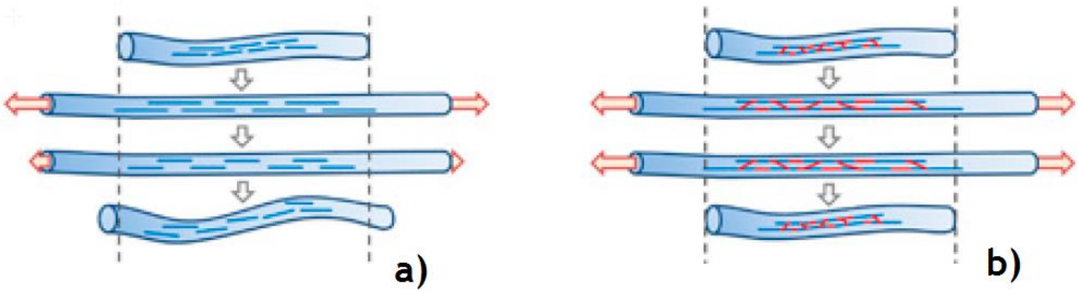


Figure 4. 3 - Difference betwen old (left) and young (right) ligaments; red lines represent elastic fibres inter-connecting the collagen fibres in blue [51].

During early stages of life the human body goes through different phases of maturation, reaching a plateau around 20 years old. In the case of ligaments, the collagen maturation results from the increase in cross-links in terms of quantity and quality. This results in an overall increase in tensile strength [45, 46].

Once maturation is achieved, the tissues will maintain their characteristics until mid-thirties. After this, the tissue degradation will start to be noticed. The most visiable effect of aging is in the skin of a person, showing wrinkles and freckles. In the case of ligaments, their tensile strength and stiffness will decrease. With aging levels of collagen naturally decrease, leading to a less resistant tissue, with collagen type I being substituted by type III. These changes will compromise the tissues abilities to sustain deformation. Other studies also report a decrease in collagen fibre diameter and an overall change in configuration [44-46].

As referred previously, in a healthy young ligament the collagen present a S shape configuration. This allows the ligament to extend without tissue damage. As the body ages, the inter and intramolecular cross-binding of the collagen fibres increases, consequently the ligament becomes stiffer. In older ligaments, the loss of elastin will compromise the fibres ability to return to its original shape. When this happens the tissue may become losse, leading to pelvic disorders such as prolapse and incontinence. Even though the collagen fibres tend to increase in strength (up to 400%), they become more fragile and the aging process causes a decrease of the tensile strength of the urogenital tissue about 60% [8].

Different studies have shown the expected effects of aging, including an overall increase of tissue stiffness [31, 33, 36]. These studies were referred in section 2.5.

4.3. Mechanical Properties

The mechanical properties are defined by interaction of the different components present in the ligaments. The tissues of the pelvic floor have a non-linear, viscoelastic behaviour, and although some tissues have similar mechanical behaviours in young women, this may not apply in later stages of life. The aged tissues may become longer, stiffer and usually more anisotropic, and this can occur naturally, not necessarily as the side effect of a trauma [36, 52]. The viscoelasticity means that the response is dependent of the strain rate. Also, when applying cyclic loads to the tissue a hysteresis phenomenon will occur, resulting in different loading and unloading curves [53].

The tissues will respond differently in stress relaxation and creep tests, due to the progressive recruitment of the fibres in the creep test [53].

From a mechanical point of view, an isotropic material responds to load independently from its direction. Biological tissue tends to show the opposite behaviour, an anisotropic response to load. A material falls in this category when the response is dependent of the direction of the load[54]. Due to its characteristics, ligaments are considered highly anisotropic materials. The fibrous nature of the material, and unidirectional orientation of its fibres, leaves ligaments resistant to tensile loads. On the other hand, they show little resistance to compression and bending tests [55]. It is the ground substance that is responsible for the response of the tissue to compressive loads [55].

Several studies, referred by Cowin and Doty (2007) [55], focused on the inhomogenety of tissues. The inhomogety of ligaments is believed to be prominent near the insertion sites, although this hypothesis is yet to be tested due to the difficulties associated with characterizing such small regions of tissue. Due to this lack of information this characteristic is ignored in modeling, for mathematical simplification.

4.3.1. Mechanical hierarchy of the soft tissues

Several studies compared the compliance of the tissues present in the pelvic floor and found out that the bladder is the least stiff, followed by the rectum, followed by the vagina which is followed by the ligaments. In younger women there is no significant difference in the compliance of the ligaments of the pelvic floor, while on the other hand, in older cases this does not apply and the broad ligament follows the vagina, the large ligament is in the middle and the Uterosacral is the most stiff among the three [36].

The results obtained by Chantereau et al [36] are presented in the following figures:

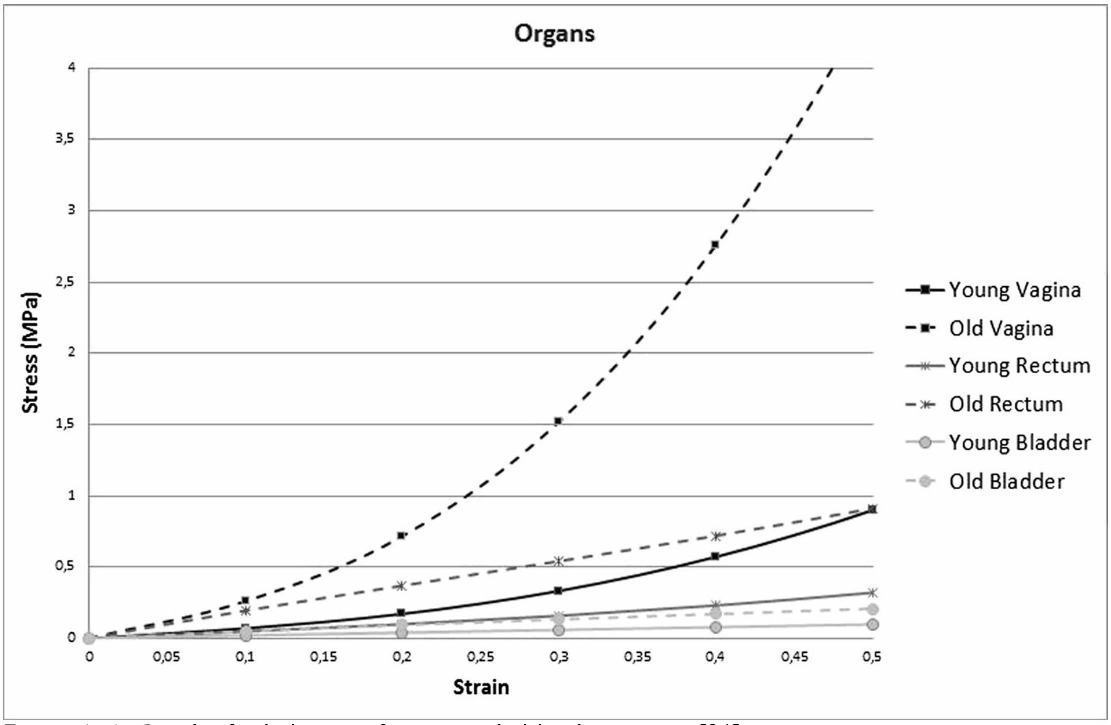


Figure 4. 4 - Results for behavior of young and old pelvic organs [36]

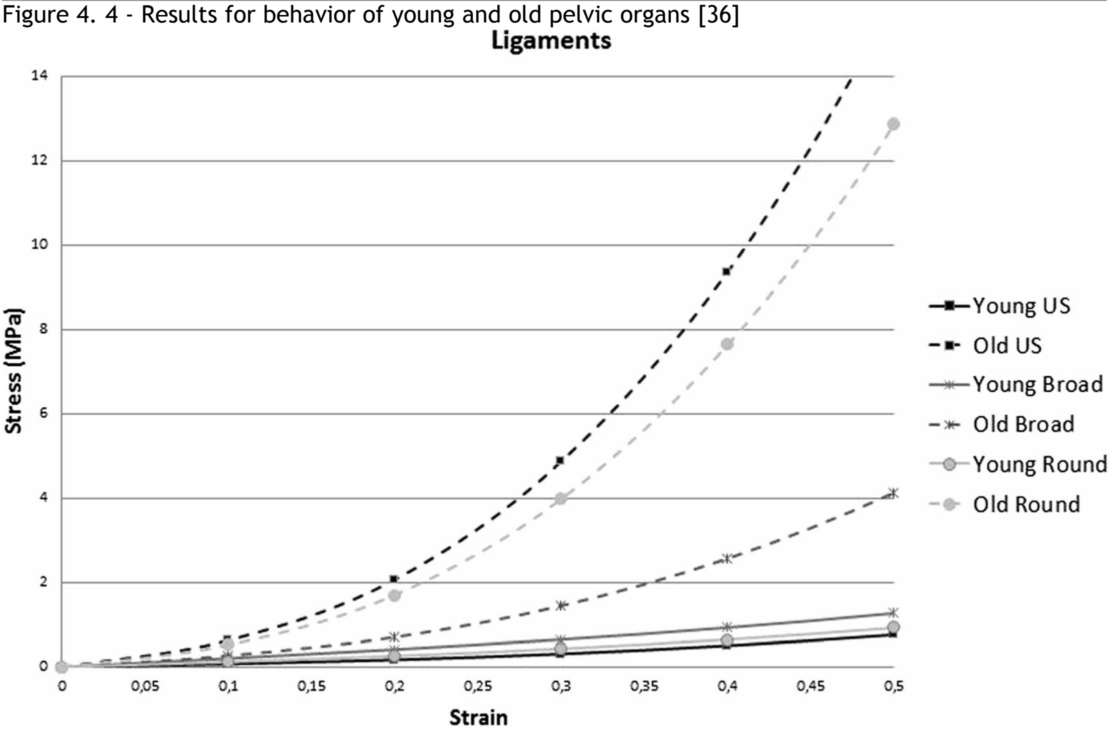


Figure 4. 5 - Results for behavior of young and old pelvic ligaments [36]

The images are a mere illustration, since further analysis and discussion of results will be over stress vs strain graphs. The results from Chantereau et al [36] show the bladder to be the least stiff organ of the pelvic cavity followed by the rectum and Vagina. As for the ligaments, the most compliant ligament is the Round ligament, followed by the Broad and USL.

Other studies report the same results regarding the behaviour of postmenopausal pelvic ligaments [33, 56].

In the case of the organs, comparing the results of young and old cases, the curve is steeper for the older meaning that for larger displacements, larger forces will be required in the older tissues.

The vagina for example shows the greatest difference when comparing both cases, this could be caused by the fact that this organ is the one to be most likely damaged in natural childbirth. The tissues once traumatized may never go back to their original state, therefore, losing the ability to respond to larger stresses, losing elasticity and becoming stiffer.

The rectum for instance shows a smaller difference in both cases, while the bladder shows an even smaller difference. This may be due to the fact that these organs don’t usually suffer trauma to the same extent as the vagina, therefore the aging process can be described as a “natural” aging of tissues with the increase of stiffness of the tissues.

In the case of the ligaments, these show similar results in the young cases and very different results in old cases. This can be caused by the fact that the ligaments are of similar constitution and in earlier stages of life they do not show evident signs of differentiation. As a woman ages the everyday standing position, possible trauma to the ligaments and the aging process itself, tend to make the ligaments stiffer and the different roles they play in the pelvic cavity differentiates them into the behaviour seen in the Figures 4.1 and 4.2 [36].

Comparing the results for the organs, the bladder proves to be the most elastic organ, as suspected, since this organ stores the urine. The rectum also stores the feces, but this is just the end of the large intestine, therefore its elasticity is justified but to some extent, since its supposed to contract and expel the feces, which are solid and should require higher pressures when compared to the urine.

Chapter 5.

Biomechanics of soft tissue

This section was introduced with an aim to present the concepts, laws and biomechanic models, which are applied in the context of soft tissues. Every step of the procedure will be included, starting by understanding the response of a tissue when it is exposed to a force, translating the relation between force and displacement (parameters obtained by the equipment), further translating those to stress and stretch.

Further, some hyperelastic constitutive models will be introduced.

The models are built based on parameters of the material, and those are defined based on the strain-energy function (Ψ). On the other hand, this will describe the mechanical behavior of the material in terms of energy [57].

Holzapfel presents hyperelastic models, representing this energy in several ways to express elastic properties of incompressibility [57]. In here we will talk of incompressible materials, since ligaments can be considerably deformed, whithout any major alteration in their volume. This behaviour is tipicall for biological soft tissue, as well as, for rubbers.

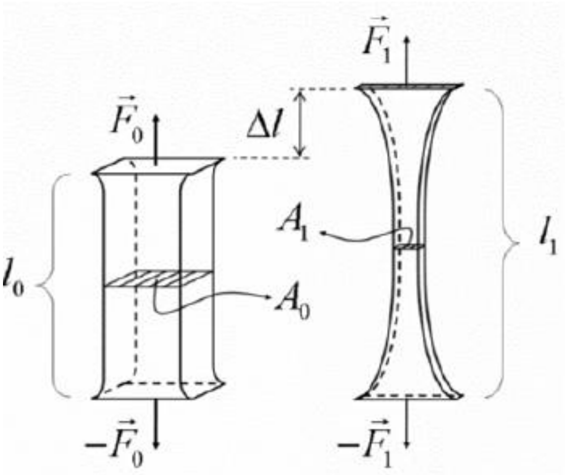
Among other models used to model hyperelastic behaviour of materials, the Ogden, neo-Hookean and Mooney-Rivlin models will be focused here. They were originally creadted to industry purposes, representing a first approach to modelling of non-linear behaviour expressed by materials, later on applied to the biological tissues. Nowadays these models are the pre-set present in simulation programs based on the finite elements model (ABAQUS, ANSYS, among others).

5.1. Mechanical Behaviour of materials

When studying the mechanical behaviour of a material, one must not forget that apart from the response of the material to a force, all types of materials are subjected to the same laws of physics. Since this report tries to focus the behaviour of soft tissues, the concepts presented will be of non-linear mechanics and materials with a hyperelastic behaviour.

The process starts on the test table in which the materials are subjected to uni or biaxial traction tests. The equipment will express the data collected regarding the force applied and displacement/enlongation Δl.

With these two parmeters, and collecting the geometrical parameters, it is possible to translate the data to stress and stretch. The stress is represented by σ and can be expressed as, $\sigma = \frac{P}{A}$(Figure 6.1). As for the deformation, thiss will be related the the initial and final length of the test sample.



A_0- initial transverse area

A_1- deformed transverse area

l_0- initial lentgh

l_1- deformed lentgh

Figure 5. 1 - Representation of the behaviour of a material during a traction test[58].

$$\varepsilon = \frac{\Delta l}{l_0} = \frac{l_1 - l_0}{l_0} \qquad 5.1$$

In the specific case of soft tissues, it is known that these show a non-linear mechanical behaviour, which mean the stress (σ) will be expressed as a function of stretch (λ), such as σ = f(λ).

The stretch (λ) can be obtained using the relation between stretch and deformation.

$$\lambda = \frac{l_1}{l_0} \qquad 5.2$$

$$\varepsilon = \frac{\Delta l}{l_0} = \frac{l_1 - l_0}{l_0} = \lambda - 1 \qquad 5.3$$

QED:

$$\lambda = \varepsilon + 1 \qquad 5.4$$

Soft tissues are considered incompressible, since they have a large percentage of water in their constitution. For this reason, during uniaxial traction tests, the alteration in volume is minimal.

When any material is subjected to a stress it will deform, and if the applied force is sufficient, the deformation caused may be permanent. In the graph of stress vs. stretch it is possible to observe that point of no return, this corresponds to the stress necessary to apply a plastic deformation to the material. The two areas divided by that point are elastic and plastic region.

In an elastic scenario, the stress applied deforms the sample but as soon as the force is removed, the material has the capacity to return to its original state. On the other hand, when the elastic limit is achived, we enter the plastic domain and in this area the material is sustaining permanent deformation, it may recover partially, but never return to its original state. The figure below shows the graph of stress vs stretch for a metal and a soft tissue, and the differences are evident (Figure 5.2).

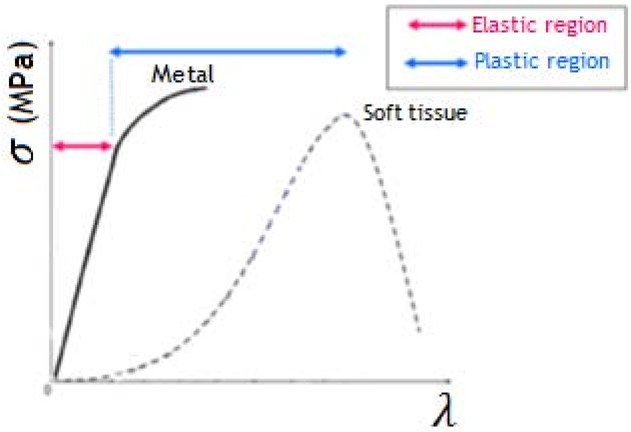


Figure 5. 2 - Stress response in a metal and a soft tissue.

The linear part of the graphic, present for both materials, is part of the elastic area and shows a direct proportionality between stress and stretch. In this part it can be said that:

$$\sigma = E\varepsilon \qquad 5.5$$

In which σ represents the stress applied to the material, E is the Young's modulus and ε is the strain.

Zooming in the graph of a soft tissue, it is clearer to see that the plastic deformation starts from the yielding stress point represented by σ_y in Figure 5.3. Passing the yielding point, the material will suffer a plastic deformation, and from this point on the linear behaviour is lost, in both soft tissue and metal.

In the soft tissue there can be considered two elasticity modulus considered: secant (*Es*) and tangent (*Et*). The *Et* corresponds to the linear zone of the graph, as for the *Es*, this is the non-linear zone, in which very little forces are needed to provoke large dissentions. In the linear zone it can be said that Hookes law is applicable, but not valid to characterize the soft tissue behaviour.

The yielding stress (σ_y) is used to define the *Et*, since this is the last point to be considered for the line. As for the *Es*, this one serves as a reference for the initiall response of the material.

$$E_s = \frac{\Delta\sigma_1}{\Delta\lambda_1} \qquad 5.6$$

$$E_t = \frac{\Delta\sigma_2}{\Delta\lambda_2} \qquad 5.7$$

The energy absorbed by the material during the deformation can be calculated as the area below the curve in the stress-stretch graph, from the initial point to the maximum stress value (σ_{max}) and final stretch (λ_U). The energy density is given by [53]:

$$U_s = \int_0^{\varepsilon_U} \sigma d\varepsilon = \int_0^{\lambda_U} \sigma d\lambda \qquad 5.8$$

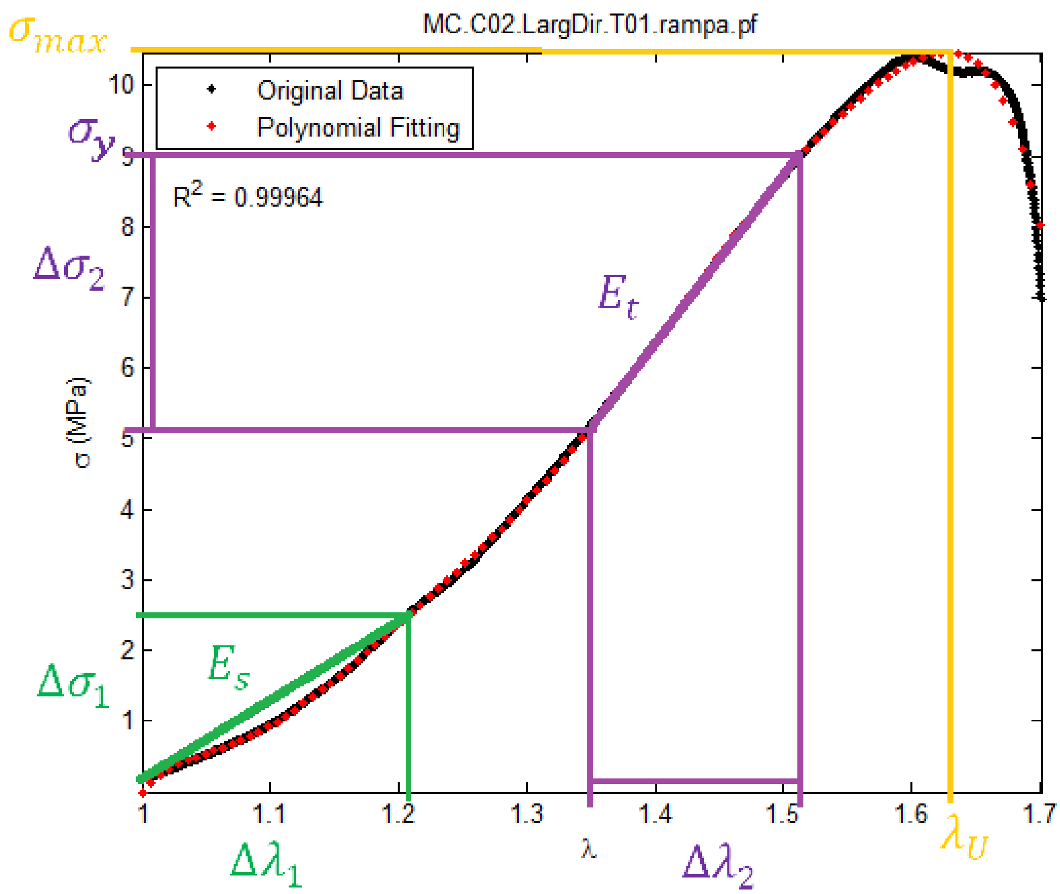


Figure 5.3- Stress vs Strech example graph

5.1.1. Constitutive models

The constitutive laws will establish the relation between stress and stretch, being the relation between these two dependent of the type or class of material. The main objective is to develop mathematical models capable of representing the real behaviour of materials [53].

This work focuses the behaviour of materials in a hyperelastic context, in other words, a non-linear behaviour, where the verified stresses can be derived from a function of elastic energy stored in the material [53].

This section will focus the study of constitutive equations in a non-linear perpective, therefore studying the typical behaviour of soft biological tissues.

5.1.1.1. Hyperelasticity

The Helmoholtz energy can be described as a function of energy of deformation, $\psi = (\mathrm{F})$, in which "F" can refer to a tensor. This is an example of a scalar function assuming "F" as a continuos variable tensor. Considering that we are talking of a homogeneus material, the energy function can be obtained through assumptions of simetry, thermodinamis and energy. In this case the function ψ will depend only of the deformation gradient F, assuming it as continuous. If the material is heterogeneous, the function ψ will depend on the point to be analised [53].

Hyperelastic materials are considered a subclass of elastic materials. In these the response function ($\mathfrak{B}$) as expressed [53]

$$\boldsymbol{P} = \mathfrak{B}(\mathbf{F}) = \frac{\partial \psi(\mathbf{F})}{\partial F} \text{ ou } P_{aA} = \frac{\partial \psi}{\partial \mathrm{F_{aA}}} \tag{5.9}$$

Having the Cauchy stress tensor as the inverse of the relation of $\sigma = \mathrm{JPF^{-T}}$:

$$\sigma = J^{-1}\boldsymbol{P}\boldsymbol{F}^{T} = \sigma^{-1} \tag{5.10}$$

Assuming, $\mathrm{J} = \det \mathrm{F}$, we obtain:

$$\sigma = g(\boldsymbol{F}) = J^{-1}\frac{\partial \psi(\mathbf{F})}{\partial \boldsymbol{F}}\boldsymbol{F}^{T} = J^{-1}\left(\frac{\partial \psi(\mathbf{F})}{\partial \boldsymbol{F}}\right)^{T} \tag{5.11}$$

or

$$\sigma_{ab} = J^{-1}F_{ab}\frac{\partial \psi}{\partial \mathrm{F_{Ab}}} = J^{-1}F_{ab}\frac{\partial \psi}{\partial \mathrm{F_{bA}}} \tag{5.12}$$

These equations are an example of state equations, or constitutive equations, in which it is establish an empirical model or axiomatic base on the approximation of the behaviour of the real material [57].

The gradient of ψ, obtaind through the derivation of the scalar function ψ in order to F, is a second order tensor, in this case corresponding to the first Piola-Kirchhoff, P. By derivation the component function $\psi(\mathrm{F_{aA}})$, we ensure that is differentiable for all components of F_{aA} [53].

The energy function, converging, tends to zero in the reference configuration, in other words [53], when $F = I$:

$$\psi = \psi(\mathbf{I}) = 0 \tag{5.13}$$

Take notice that the strain-energy function ψ increases as the deformation itself increases, therefore it is safe to conclude that:

$$\psi = \psi(\mathbf{F}) \geq 0 \tag{5.14}$$

This will limit the limit the comparation in between expression for deform energy, so we must assume that ψ has no static points when deformed.

Equations 5.13 and 5.14 reffer to the residual stresses, so we assume that the initial energy as zero, meaning that the reference configuration is stress free.

When reffering to finite deformation a function must repesct the following restrictions:

$$\psi(\mathrm{F}) \rightarrow +\infty \qquad se \qquad \det \boldsymbol{F} \rightarrow \infty \tag{5.15}$$

$$\psi(\mathrm{F}) \rightarrow +\infty \qquad se \qquad \det \boldsymbol{F} \rightarrow 0^{+} \tag{5.16}$$

From a phisycs perpective, this means that in order to continuoslhy expand the body to infinity, or reduce it to almost zero, it is needed an infinite amount of energy [53] [[57].

5.1.1.2. Incompressible hyperelastic materials

A material is considered as incompressible when it can deform without volume alterations. A good example of these materials are polymers [53], in which:

$$J = 1 \tag{5.17}$$

The material is considered incompressiable when it is subjected to an internal restriction. In order to derivate constitutive equations to these materials, the function of strain-energy function will establish [53]:

$$\psi = \psi(F) - p(J - 1) \tag{5.18}$$

In this case the strain-energy function , ψ, will be defined by: $J = \det \boldsymbol{F} = 1$. The constant p is a scalar defined as the Lagrange multiplier, it can also be considered as the hydrostatic pressure. These parameters can only be calculated based on the equilibrium equations of the boundary conditions [53].

To calculate the constitutive general equantions for the first Piola-Kirchhoff stress tensor, P, we need to derivate equatios 5.18 in order of the deformation gradient, F, using the identity $\frac{\partial \mathbf{J}}{\partial \boldsymbol{F}} = JF^{-T}$ [53]:

$$\boldsymbol{P} = -p\boldsymbol{F}^{-T} + \frac{\partial \psi(\mathbf{F})}{\partial \boldsymbol{F}} \tag{5.19}$$

Multiplying the previous equation (5.19) for F^{-1} on the left, it is possible to obtaine the second Piola-Kirchhoff stress tensor [53], S:

$$\boldsymbol{P} = -p\boldsymbol{F}^{-1}\boldsymbol{F}^{-T} + \boldsymbol{F}^{-1}\frac{\partial \psi(\mathbf{F})}{\partial \boldsymbol{F}} = -p\boldsymbol{C}^{-1} + 2\frac{\partial \psi(\mathbf{C})}{\partial \boldsymbol{C}} \tag{5.20}$$

In which $\mathbf{C} = \mathbf{F}^{\mathrm{T}}\mathbf{F}$ and $\mathbf{C}^{-1} = \mathbf{F}^{-1}\mathbf{F}^{-\mathrm{T}}$.

If the previous equation (5.20) is multiplied on the right for F^T, knowing that $\sigma = J^{-1}\boldsymbol{P}\boldsymbol{F}^T = \sigma^{-1}$, we obtain the Cauchy-Green stress tensors, T, which is expressed by [53]:

$$\sigma = -p\boldsymbol{I} + \frac{\partial \psi(\mathbf{F})}{\partial \boldsymbol{F}}\boldsymbol{F}^T \equiv \sigma = -p\boldsymbol{I} + \boldsymbol{F}\left(\frac{\partial \psi(\mathbf{F})}{\partial \boldsymbol{F}}\right)^T \tag{5.21}$$

The equations 5.19, 5.20 and 5.21 are three fundamental constitutive equations, and are used as a general way to define incompressible hyperelastic materials in finite defornations.

In cases of isotropy the dependence of ψ for the Cauchy-Green tensors C and B^*, is expressed by the tree tensor invariants $\psi = \psi[I_1(C), I_2(C), I_3(C)] = \psi[I_1(B^*), I_2(B^*), I_3(B^*)]$. In cases of incompressibility the third invariant is [53]:

$$I_3 = \det C = \det B^* = 1 \tag{5.22}$$

However the first and second invariants are variables for independent deformations. As so, the fuction for strain-energy function for incompressible hyperelastic materials is [53]:

$$\psi = \psi[I_1(C), I_2(C)] - \frac{1}{2}p(I_3 - 1) = \psi[I_1(B^*), I_2(B^*)] - \frac{1}{2}p(I_3 - 1) \tag{5.23}$$

In which $\frac{p}{2}$ corresponde to Lagranges indetermined multiplier and the invariants $I_1 = \lambda_1^2 + \lambda_2^2 + \lambda_3^2$ and $I_2 = \lambda_1^2\lambda_2^2 + \lambda_2^2\lambda_3^2 + \lambda_3^2\lambda_3^2$.

To verify the previous equations in terms of the first and second tensor invariants, the expression can be derived in order to the tensor C:

$$S = 2\frac{\partial\psi(\mathrm{I}_1, I_2)}{\partial C} - \frac{\partial[\mathrm{p}(\mathrm{I}_3 - 1)]}{\partial \mathrm{C}} = -pC^{-1} + 2\left(\frac{\partial\psi}{\partial I_1} + I_1\frac{\partial\psi}{\partial I_2}\right)I = 2\frac{\partial\psi}{\partial I_2}C \tag{5.24}$$

Which translats to the constitutive equation: $S = 2\frac{\partial\psi(\mathrm{C})}{\partial C} = 2\left[\frac{\partial\psi}{\partial I_1} + I_1\frac{\partial\psi}{\partial I_2}\right]I - \frac{\partial\psi}{\partial I_1}C + I_3\frac{\partial\psi}{\partial I_3}C^{-1}$, in which we can replace $I_3\frac{\partial\psi}{\partial I_3}$ por $\frac{p}{2}$.

5.1.1.3. Constitutive models for incompressible materials

Ogden Model

This model was first introduced by R. W. Ogden in 1972, presenting us with great versatility being capable of capturing the non-linear behaviour of several materials, with a good correlation for higher deformations [53]. The model will base the free energy in the main relative enlongments $(\lambda_1, \lambda_2, \lambda_3)$:

$$\Psi^{Ogden}(\lambda_1, \lambda_2, \lambda_3) = \sum_{p=1}^{N} \frac{\mu_p}{\alpha_p}\left(\lambda_1^{\alpha_p} + \lambda_2^{\alpha_p} + \lambda_3^{\alpha_p} - 3\right) \tag{5.25}$$

- adimensional constant

N- Number of terms (positive) α_p - adimensional constant μ_p- shear module

N

μ_p

α_p

Comparing with the linear theory, the following condition is obtained:

$$2\mu = \sum_{p=1}^{N} \mu_p\alpha_p \text{ com } \mu_p\alpha_p > 0, p = 1, \ldots, N \tag{5.26}$$

In which the parameter μ represent the classic shear modulus in the reference configuration, obtained from the linear theory.

Neo-Hookean Model

This model can be studied as a specific case on the Ogden model, in which $N = 1$ and $\alpha_p = 2$ [53]. The function will depend only on the first tensor invariant:

$$\Psi^{Neo-Hookean} = c_1(\lambda_1^2 + \lambda_2^2 + \lambda_3^2 - 3) = c_1(I_1 - 3) \qquad 5.27$$

Modelo de Mooney-Rivlin

This model can also be studied as a specific case of the Ogden model, in which $N = 2$ and $\alpha_1 = 2$ and $\alpha_2 = -2$.

Though the first to invariants, I_1 and I_2, with $I_1 = \lambda_1^2 + \lambda_2^2 + \lambda_3^2$ and $I_2 = \lambda_1^2\lambda_2^2 + \lambda_2^2\lambda_3^2 + \lambda_3^2\lambda_3^2$ and with the condition $I_1 = \lambda_1^2\lambda_2^2\lambda_3^2 = 1$. This way the function of strain-energy function for the model is given by [53]:

$$\Psi^{\mathrm{Mooney-Rivlin}} = c_1(\lambda_1^2 + \lambda_2^2 + \lambda_3^2 - 3) + c_2(\lambda_1^{-2} + \lambda_2^{-2} + \lambda_3^{-2} - 3) \qquad 5.28$$

$$\equiv \Psi^{\mathrm{Mooney-Rivlin}} = c_1(I_1 - 3) + c_2(I_2 - 3) \qquad 5.29$$

In which c_1 and $c_2$represent the materials constants, subjected to the following restrictions:

$$c_1 = \frac{\mu_1}{2} \text{ e } c_2 = -\frac{\mu_2}{2} \qquad 5.31$$

From the previous equation (5.31) the shear model is obtained with the valyue of $\mu_1 - \mu_2$. For a better approximation for the non-linear behaviour, the constants c_1 and c_2 must be optimized to:

$$c_1 > 0 \text{ e } c_1 + c_2 = 0 \qquad 5.32$$

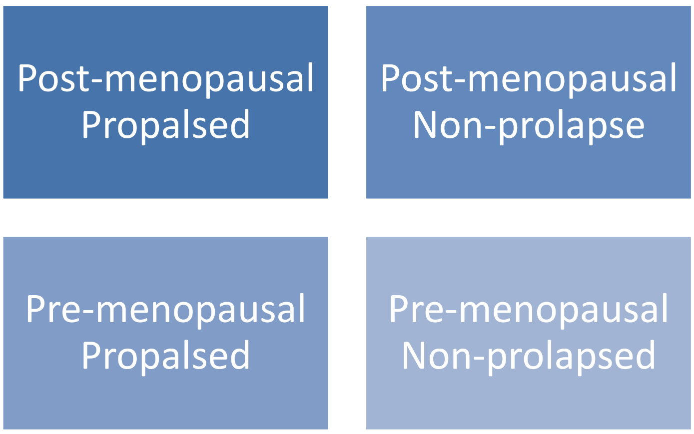
This model is taken as precise to model the non-linear behaviour of isotropic materials, such as rubbers [53].

Chapter 6.

Methodology

6.1. Introduction

This chapter was introduced to specify all the data processing involved in acquiring the experimental data, from the preparations of the test sample to the analysis and data processing. To understand the mechanical properties of the pelvic ligaments and the changes associated with aging and pelvic prolapse, four groups where established figure 7.1). This study was conducted following the Ethical Research Ethics Committee guidelines of Hospital São João, including the previous consent provided by all the patients involved in the investigation. All patients were asked to fill questionnaires to gather information regarding their medical history. This included the number of childbirths (and type), prolapse, UI and FA incontinence history.



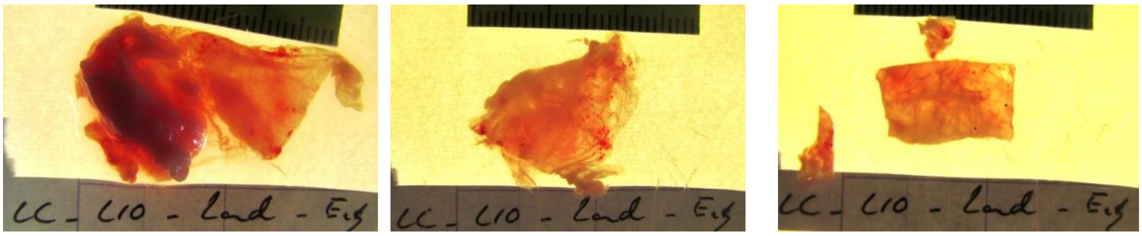
The case group admitted in this project fills in two groups of the initially proposed four; those were non-prolapsed and post-menopausal women and non-prolapsed and pre-menopausal women. Even though samples from prolapsed women were collected, the samples were too small for the mechanical testing. Since the cases presented refer only to post- and pre-menopausal women, the prolapse variable will be omitted from here on.

Inclusion criteria for the prolapsed group consists on women who were submitted to pelvic prolapse correction surgery and the tissues were surgical left-overs collected for mechanical testing. As for the control and menopausal subjects, these were cases also submitted to hysterectomy, but due to preventive surgery in cancer related scenarios.

6.2. Tissue samples of pelvic ligaments

The samples were collected during hysterectomy surgeries and stored in saline solution. They were transported to INEGI in a refrigerated container and the tests were performed in no more than 6 hours post-surgery.

The main direction of the fibres was identified by constraining the sample between two glass plates and/or observing the main fibre direction using a light table. Depending on the size of the sample, the number of possible tests was variable. Up to date only two tests were performed from the same sample. This is due to the fact that the samples are small and the preparation requires cutting the tissue. The specimens were cut in rectangular shape.



a) b) c)

Figure 6. 2 – Example of pelvic ligament sample and different stages of preparation; a) Received tissue sample; On the right the sheet-like fibrous tissue (ligament), on the right other tissues (blood vessels, muscular tissues); b) Isolation of the target tissue; c) Final samples, prepared for tests.

6.3. Geometrical analysis

Geometrical parameters were acquired in two different ways. The thickness was measured by placing the sample between two glass plates and measuring the whole set, subtracting the thickness of the glass. The length and width were acquired using ImageJ.

Figure 6. 3 – Digital Caliper used to measure the thickness of the samples and glass plates.

6.4. Stiffness and maximum stress assessment

The biomechanical characterization of the tissues consisted of performing uniaxial tension tests. The tests performed are considered ex-vivo, since the tissues are collected and extracted from a living organism and the rigor mortis has not settled in. The preservation is guaranteed by the transport and storage of the samples in cold temperatures. To perform the tests, the samples are secured on the testing machine using metal grips. The possibility of using intermediary interface between the metal and the specimen was tried, however the fragility of the ligaments made the securing of the sample more difficult. This should be used in the case of less fragile tissues, but with pelvic ligaments we opted not to use them. This allowed for better sensibility when gripping the sample.

The testing machine is a custom-made designed to work with soft tissues. The load (N) and displacement (mm) data were acquired using a load cell and two actuators. The testing protocol was based in other studies [33, 59],[60]. Due to the size of the specimens the protocol consisted on a simple creep with pre-conditioning of the sample. The prepared specimen was mounted on the machine and the pre-load was applied. Next the pre-conditioning of the sample was performed, consisting of 5 cycles between 0.25 and 0.5 N. This step was not always exact, due to the sensors accuracy. After this step the samples were stretched at a constant displacement rate of 5 mm/min. The actuators have a 120 N load limit and the load cells and have a maximum load of 45 N mark. The process was recorded using a digital camera positioned directly above the sample. This was used for quality control of the tests for a posterior elimination of tests where slippage was detected.

The mechanical parameters presented in this report are the ligaments maximum load, maximum displacement, tensile strength, maximum stretch, strain energy density (SED), yield strength. The discussion of the results will be focused on the tensile strength and the maximum stretch.

The objective of the project is to study and compare the biomechanical properties of pelvic ligaments between pre- and post-menopausal women, as well as, POP and non-POP women and the comparison between the orientations of the fibres during the test. As referred previously, up to date, only cases of two of the initially considered 4 groups were studied. Therefore, the main objective in this report is to compare the biomechanical properties of pelvic ligaments between pre- and post-menopausal women, as well as, measure the influence of the main orientation of the fibres. This comparison was based on the analysis of the mechanical parameters extracted from the biomechanical tests.

6.5. Experimental data filtering

The data collected by the testing machine (Load (N) and Displacement (mm)) was filtered using a MATLAB script, previously created by Pedro Martins. This script was DataFilter.m, and convoluted the data in order to smooth the experimental data. It also allowed to reset both load and displacement sensors, as well as select the last point to consider. A print screen was included in in figure X. This script had been previously used by Martins et al. (2013) [33].

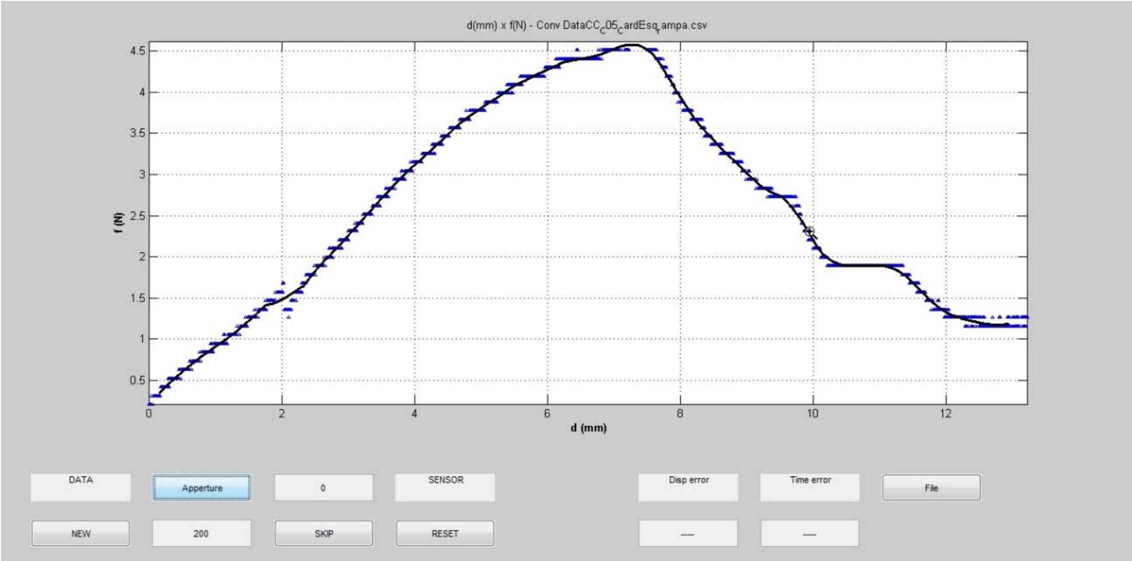


Figure 6. 4- DataFilter sctript example used in the data filtering of the uniaxial tension tests performed

The acquisition frequency used was 100Hz. The blue points represented in figure 6.1 are the collected raw data extracted from the acquisition software and they were convoluted with an aperture defined by the user. This allowed a suavization of the curve, leading to a better modelling of the behaviour.

The resulting curve was exported to a .dat file, which also saved the geometrical parameters of the sample

6.6. Experimental data analysis

The data processing was performed with the use of dPrevdPreoc.m matlab script. A printscreen is demonstrated in figure 6.2. The script displayed the geometrical parameters and allowed the plot of three different types of graphics and the automatic calculus of its mechanical properties. The calculations associated with each parameter are assessed in section 5.1.The three types of graphics were:

<u>Force vs Displacement</u>

- Load max (N) - maximum load recorded (F_{max})

- Disp max (mm) – maximum displacement recorded (d_{max})
- Work of F (mJ) – area below the graphic curve (W_{force})

<u>Stress vs Stretch</u>

- Tensile strength (MPa) – maximum stress recorded (σ_{max})
- Ultimate stretch – maximum stretch recorded (λ_{max})
- Energy density$(\frac{mJ}{m^3})$ – area below the graphic curve U_{stress}

<u>Stress vs Strain</u>

- Tensile strength (MPa) – maximum stress recorded (σ_{max})
- Ultimate strain – maximum strain recorded (ε_{max})
- Strain energy (SED) – area below the graphic curve Ψ

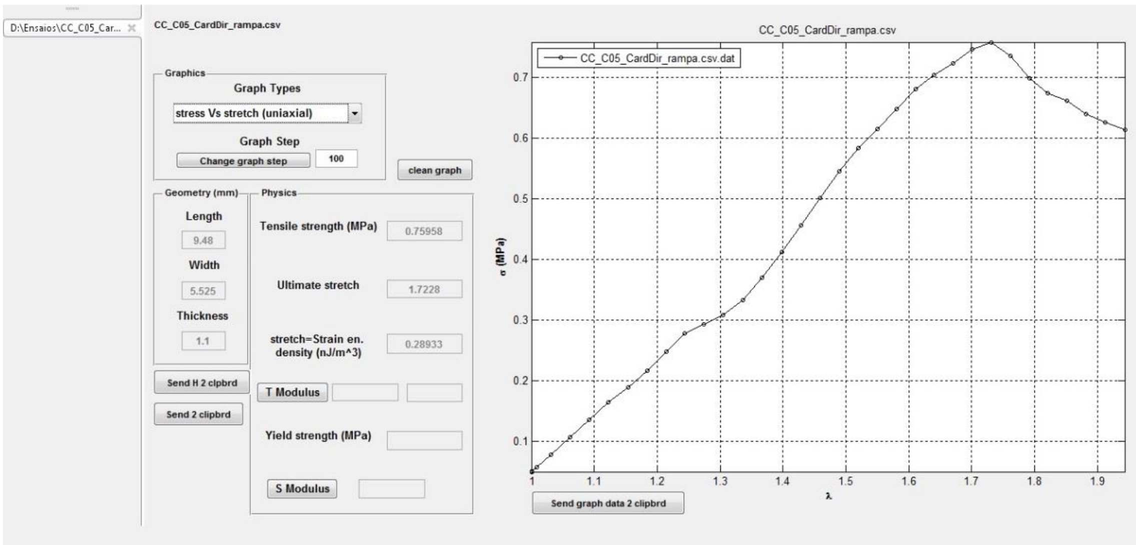


Figure 6. 5- dPrevdProc sctript example used for the data analysis

6.7. Statistical Analysis

The parameters collected with dPrevdProc.m are stored in an excel file and the mean values are calculated. For better comparison and visualisation of the different curves, another matlab script was used, SuperFit.m. The data used in this step was extracted of dPrevdProc, by selecting the type of graph wanted and using the "send2clipboard" button. The data was saved in a .txt file and analysed using SuperFit. As proved by figure 6.3 this simplified the selection and validation of the samples, allowing a simple visualization of all the samples.

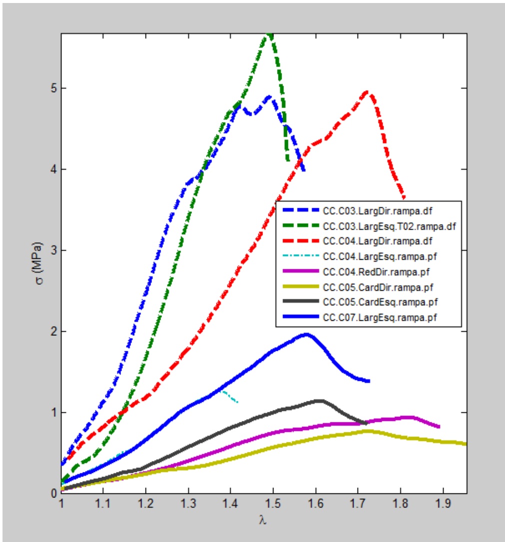


Figure 6. 6- SuperFit script example used for comparasin and validation of the results

Chapter 7.

Results and Discussion

7.1. Sample geometry

All samples which were large enough to be tested were measured, as reffered in the previous chapter. The geometric information on the specimens is presented in table 7.1. So far 9 cases were recorded, but from case 1 only one sample was received which had already been damaged. The specimens from case 6 and 9 were photographed, but the samples were too small to be tested.

The size of the samples is also related to the surgical procedure. During a vaginal hysterectomy, less excess tissue is removed (case 1 and 6). On the other hand, abdominal hysterectomy is more invasive and leads to more excess tissue removal.

Appendix A presents all the geometries of the samples. All measurements result from the average of (usually) four measurements in different positions in the sample. From left to right:

- The first column refers to the case number;
- The second column refers to the ligament;
- Third column refers to the side from which the ligaments was extracted;
- Thickness is presented in the fifth column;
- In the last two columns two values of length and width are presented. The pre-load corresponds to the measurement after the pre-load and before the pre-conditioning. The second measurement is referring to after the pre-conditioning of the sample. The third value corresponds to the difference in percentage;

As expected all samples increase in length after the pre-conditioning. This proves the hypothesis of residual stress in the sample, which will not be taken under consideration during the characterization. Hence the pre-conditioning, to prepare the sample for the tension tests.

7.2. Test validation

Previously to the analysis of the results, the tests were validated. For an easier visualization the samples were grouped in post- and pre-menopausal women. Figure 7.1 presents the stress vs stretch curves of the pre-menopausal women while figure 7.2 presents the stress vs stretch curves of the post-menopausal women.

The dashed line presented in light blue refers to the left broad ligaments from case 4, which ended prematurely, therefore this test was discarded. The specimen was tested transversely to the direction of the fibres and reviewing the recorded video, the sample ruptured from the centre. Hence the hypothesis of rupture from on the grips was discarded. The premature rupture may have been caused by bad handling of the sample, causing some trauma to the tissue. The specimen was the thickest broad ligament tested so far, this could be a sign of fibrosis or tissue damage. Even though this is pure speculation, the test was discarded until prove that this is a normal behaviour is found.

The other three dashed lines refer to the longitudinal tests performed on ligaments. The blue full line (from case 7 left broad ligament) was initially considered to be a test on the direction of the fibres, but reanalysing the graph the test was considered transversal. This test was transferred to the transversal test group, represented by the full lines. There is a clear differentiation between transversal and longitudinal tests, even though the number of samples is limited.

Although the number of post-menopausal cases was more limited, the results tend to show higher stiffness, which was expected. Initially all the tests were identified as transversal, but the clear difference of case 2 right broad ligament second test (MC_C02_Larg_Dir_T02) lead us to believe that this sample should be considered longitudinal instead. The red line refers to case 2 right broad ligament first test (MC_C02_Larg_Dir_T02) and, as seen in figure X, it ended prematurely. The sample may have ruptured from the grip, but the images are not clear. Also, since two tests were performed from one specimen, the extra handling of the tissue could have cause damage, leading to premature rupture.

The other three lines refer to the transversal tests. The light blue and purple lines, which almost coincide, are from the uterosacral ligament from case 08.

7.3. Mechanical Properties

The results presented in this report represent a small sample of tests. Even though the statistical analysis is not robust, the results start to show a tendency. The measurements were divided in pre- and post-menopausal women and grouped in transversal and longitudinal test. linear behaviour.

The specimens extracted from pre-menopausal women, tested in a perpendicular direction of the fibres (N=4), demonstrated tensile strength of 1.033±0.5 MPa (Mean ±StandD) (ranging from 0.759 to 1.953 MPa) and maximum stretch of 1.667±0.108 (ranging from 1.569 to 1.816). The tensile strength of the longitudinal test of the same group (N=3), was 4.97± 0.44 MPa (ranging from 4.889 to 5.690 MPa) and the maximum stretch was 1.495±0.133 (ranging from 1.493 to 1.725).

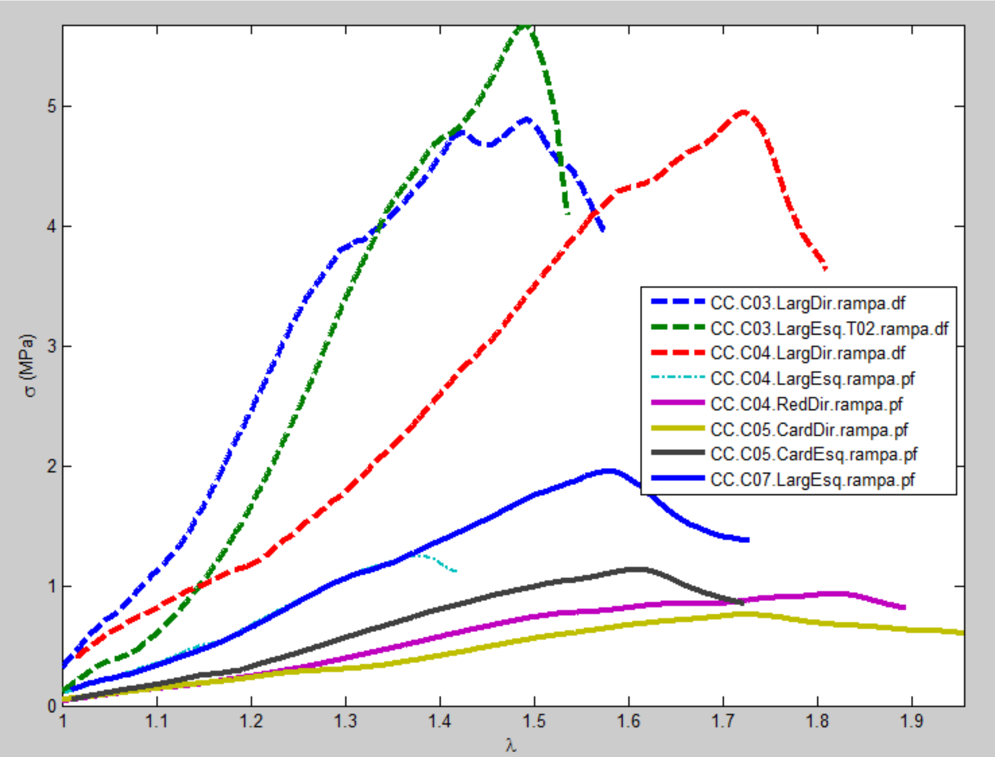


Figure 7. 1- Stress vs Stretch graphs of the samples from pre-menopausal women, longitudinal and transversal tests.

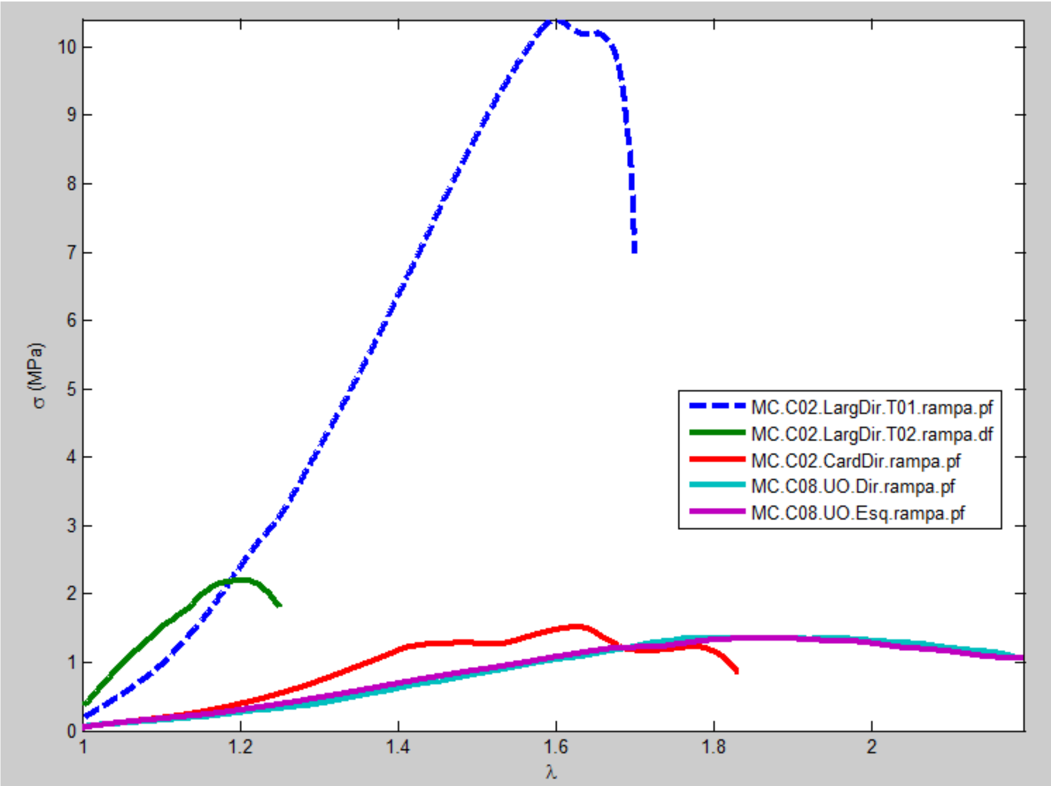


Figure 7. 2 - Stress vs Stretch graphs of the samples from post-menopausal women, longitudinal and transversal tests.

The specimens extracted from post-menopausal women, tested in a perpendicular direction of the fibres (N=3), demonstrated a tensile strength of 1.359±0.097 MPa (ranging from 1.346 to 1.521 MPa) and the maximum stretch of 1.814± 0.124 (ranging from 1.626 to 1.861). In this group only one sample was tested in the longitudinal direction. It showed a 10.381 MPa of tensile strength and 11.381 of maximum stretch.

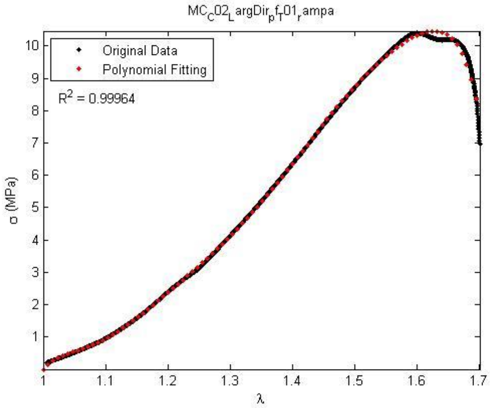


Figure 7. 3 – Stress vs Stretch graph for post-menopausal, tested in the direction of the fibres.

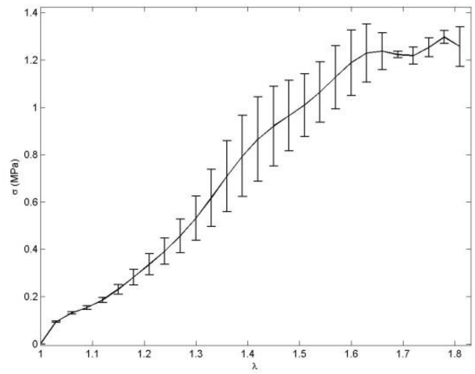


Figure 7. 4 – Stress vs Stretch graph for post-menopausal, tested perpendicular to the fibres direction; Representation of the mean curve with standard deviation.

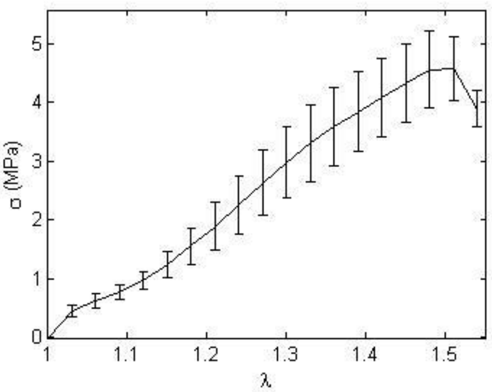


Figure 7. 5 – Stress vs Stretch graph for pre-menopausal, tested in the fibres direction; Representation of the mean curve with standard deviation.

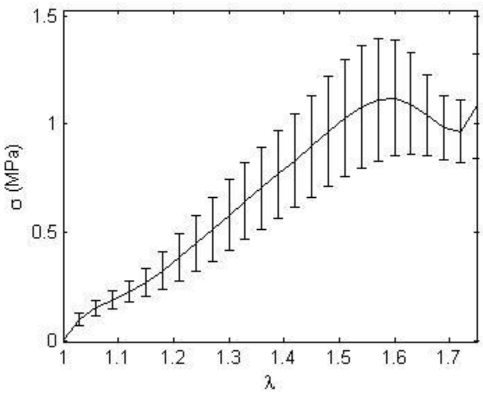


Figure 7. 6 – Stress vs Stretch graph for pre-menopausal, tested perpendicular to the fibres direction; Representation of the mean curve with standard deviation.

Figure 7.4, 7.5 and 7.6 represent the mean curve corresponding to the same results presented previously. Figure 7.3 represents the only specimen tested, so far, in the direction of the fibres in the post-menopausal group. The standard deviations fall into what was expected of the results. The fluctuations in figure 7.6 are due to the different maximum stretch recorded in different tests (as seen in figure 7.1). In figure 7.5 this does not occur since the different tested recorded a more similar behaviour. Figure 7.4 shows fluctuations in the last part of the curve affected by the behaviour of samples MC_C02_Card_Dir (blue line in figure 7.2). The last point considered for these curves was approximately the last point of the first test to rupture, the one with the lower maximum stretch.

Table 7. 1 – Analysis of pelvic ligaments mechanical characteristics divided by pre- and post-menopausal, grouped by transversal and longitudinal tests referring to the direction of the fibres.

Group	Fibres Orientation	Mechanical Properties	Tensile Strength (MPa)	Ultimate Stretch	SED	*Et*	Yield Strength (MPa)	*Es*
Pre-Menopause	Transv	Mean	1.033	1.667	0.509	2.101	0.728	1.378
		Stand	0.528	0.108	1.241	0.994	0.506	1.003
	Long	Mean	4.971	1.495	1.419	16.54	3.460	7.793
		Stand	0.441	0.134	0.262	4.915	0.386	2.701
Post-Menopause	Transv	Mean	1.359	1.814	0.542	2.188	0.983	1.499
		Stand	0.097	0.124	0.073	1.337	0.059	0.165
	Long	Mean	10.381	11.381	12.381	13.38	14.381	15.381
		Stand	-	-	-	-	-	-

7.4. Age-related changes

The statistics is not robust, since the number of tests is small. Nonetheless, the results tend to show a typical behaviour. Comparing post- and pre-menopausal results, for both directions, there is a clear increase in tensile strength (1.359 to 1.033 MPa for transversal tests and 10.381 to 4.971 MPa). The difference in the transversal tests between pre- and post-menopausal is not very significant, 1.033 MPa for pre-menopausal and 1.359 for post-menopausal. On the other hand, the results for the tests performed in the direction of the fibres the difference is very clear, 4.971 for pre-menopausal and 10.381 MPa for post-menopausal (the later value represents only one test). These results meet the same conclusions reached by two other studies [36];[56];[33]. On the other hand, the same does not apply comparing with other studies [24, 31, 46, 61].

If the results for tests on post-menopausal cases on direction of the fibres tend to be around the values reached by the sample which was tested so far. With aging the pelvic ligaments tend to become stiffer, reaching higher values of tensile strength during tension tests.

To complete the study of the changes associated with age, histochemical characterization of the ligaments would be essential, has assessed in previous studies [44, 45, 60].

7.5. Fibres orientation

As expected, fibres orientation plays a major role in tissue biomechanics [59, 62]. Higher tensile strength in longitudinal tests reveals the organized fibrous nature of ligaments. Again, the number of tests is small but the results are showing a typical behaviour. In the transversal tests, the difference is not evident. The results vary from 1.033 MPa for transversal orientation to 4.971 in the fibre oriented tests in the pre-menopausal group. On the post-menopausal group, the results were 1.359 MPa in the transversal tests to 10.381 MPa (the later value corresponds only to one test).

Even though the studied tissue was different, the results from Lynch et al. [62] reflect the obtained results. In tendons the fibres are more organized but the same principle applies. Tensile strength will be greater when the tension is applied in the direction of the fibres.

The results also suggest that the aging process is more evident in the longitudinal tests, since in the transversal tests the difference in results is not as evident comparing pre- and post-menopausal.

Chapter 8.

Conclusions and Future work

The results obtained indicate that fibre orientation and hormonal changes do play a major role in the mechanical behaviour of pelvic ligaments. For instance post-menopausal ligaments tend to show higher tensile strength, as was expected. Ideally the characterization should be performed in the direction of the fibres. This is not always possible, as the specimens obtained are surgical left-overs.

The tests performed in the transversal direction of the fibres proved to be important, as the difference between post- and pre-menopausal is not as evident as in the tests where the tension is applied in the direction of the fibres. The difference in the later may be related with time- and history-dependent properties of ligaments. Once again, the number of samples tested limits the conclusions.

Future work will be focused in increasing the numbers of tests, to fill the other groups, as well as assess different ligaments individually. Studies have shown different aging rhythms in different ligaments [33, 36], suggesting different solicitations for different ligaments throughout life. Histo-chemical characterization of the tissue will improve the analysis of the tissue, as it will give extra information to a better characterization of the tissues.

In conclusion, aging and fiber orientation have major effects on the mechanical response of pelvic ligaments. However, no solid conclusion can be taken as the number of specimens is too small for a correct representation of the group in study.

Appendices

Appendix A - Geometrical Data

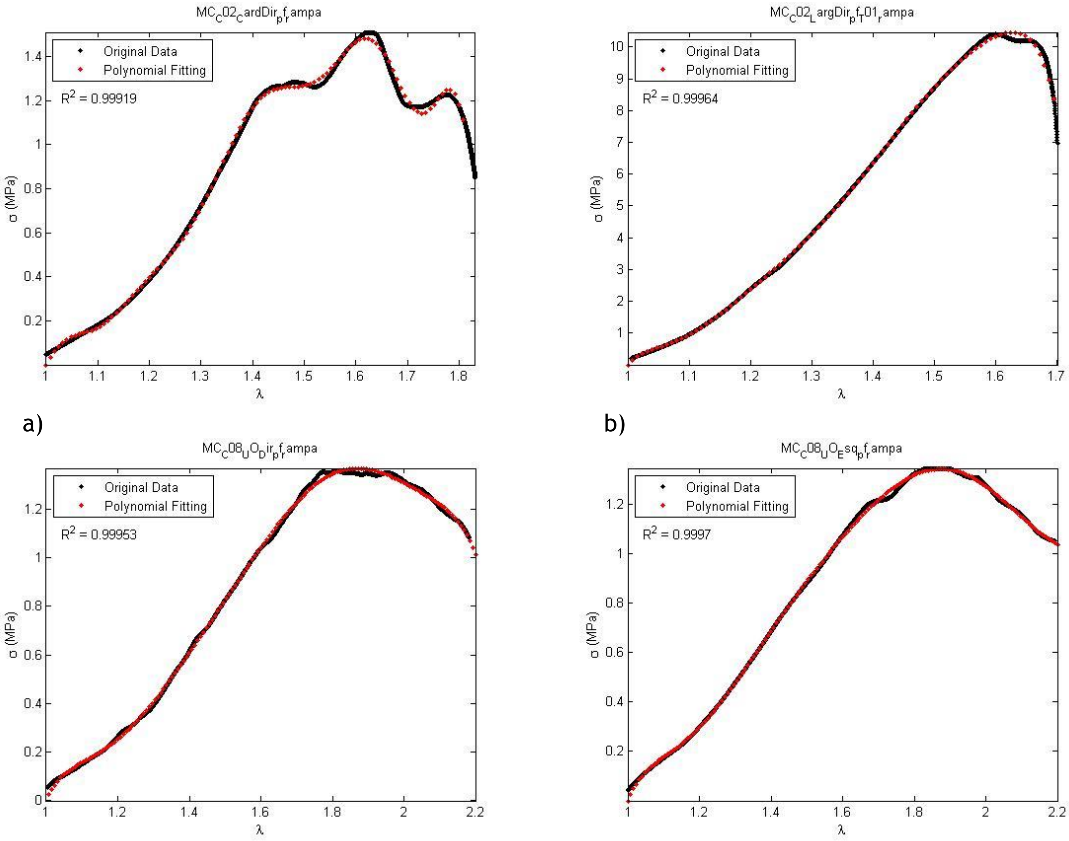
Table A. 1 – Geometrical data from all the tested specimens; first column refers to the case number; second column refers to the ligament; Third column refers to the side from which the ligaments was extracted; Thickness is presented in the fifth column; the last two columns two values of length and width are presented. The pre-load corresponds to the measurement after the pre-load and before the pre-conditioning. The second measurement is referring to after the pre-conditioning of the sample. The third value corresponds to the difference in percentage;

				Thickness		**Length**	**Width**
C02	Cardinal	Left		0.635		11.2	3.2
C02	Cardinal	Right		0.98	Pre-Load	11.26	5.855
					Pre-Cond	11.675	5.66
						4%	-3%
C02	Broad	Right	T01	0.475	Pre-Load	8.02	2.975
					Pre-Cond	8.335	2.605
						4%	-12%
C02	Broad	Right	T02	0.305	Pre-Load	8.659	1.865
					Pre-Cond	9.25725	2.08
						7%	12%
C03	Broad	Left		0.335	Pre-Load	11.115	5.55
					Pre-Cond	11.95	5.675
						8%	2%
C03	Broad	Right		0.245	Pre-Load	17.5	4
					Pre-Cond	18.44	4
						5%	0%
C04	Round	Right		1.14	Pre-Load	10.54	5.975
					Pre-Cond	10.81	5.88
						3%	-2%
C04	Broad	Left		0.625	Pre-Load	16.75	4.515
					Pre-Cond	17.24	4.985
						3%	10%
C04	Broad	Right		0.415	Pre-Load	12.21	2.7
					Pre-Cond	14.44	2.355
						18%	-13%
C05	Cardinal	Left		1.115	Pre-Load	11.85	6.08
					Pre-Cond	12.105	5.83
						2%	-4%
C05	Cardinal	Right		1.1	Pre-Load	9.045	5.525
					Pre-Cond	9.48	5.525
						5%	0%

Table A. 2 – Continuation of table A.1.

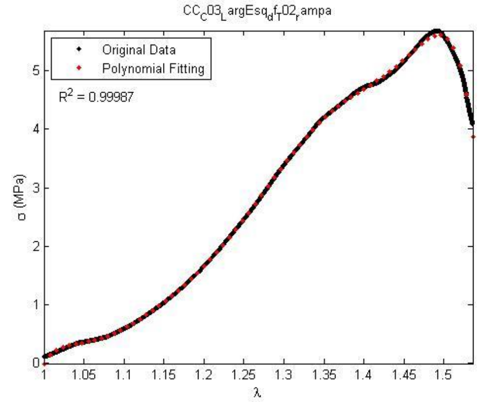
			Thickness		Length	Width
C07	Broad	Left	0.59	Pre-Load	7.56	4.25
				Pre-Cond	8.065	3.935
					7%	-7%
		Right	0.47	Pre-Load	13.485	5.425
				Pre-Cond	14.71	5.3
					9%	-2%
C08	UO	Left	0.85	Pre-Load	9.64	6.12
				Pre-Cond	10.4	6.05
					8%	-1%
		Right	1.055	Pre-Load	16.17	5.13
				Pre-Cond	17.68	5.31
					9%	4%

Appendix B - Stress vs Stretch curves

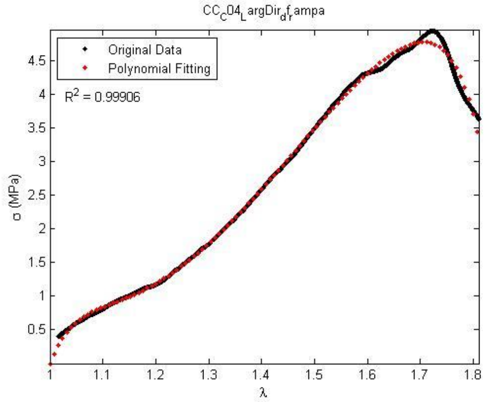


c)

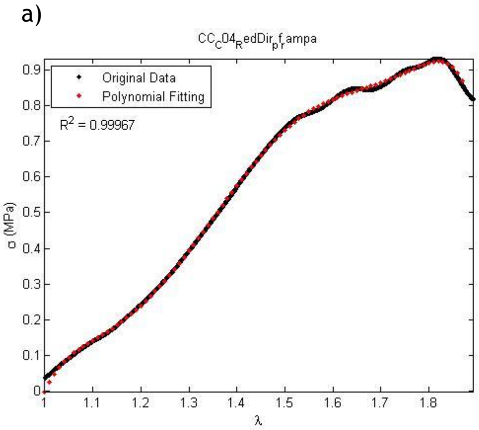
Figure B. 1 – Stress vs stretch curves for post-menopausal specimens; Representation of polynomial fitting using SuperFit.m; a) Right Cardinal ligament from Case 02; b) Right Broad ligament from Case 02; c) Right Utero-Ovarian ligament from Case 08; d) Left Utero-Ovarian ligament from Case 08.



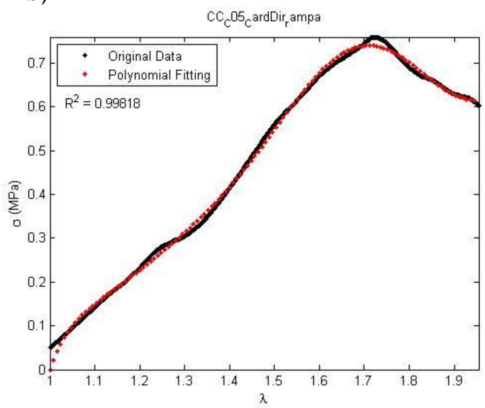
a)



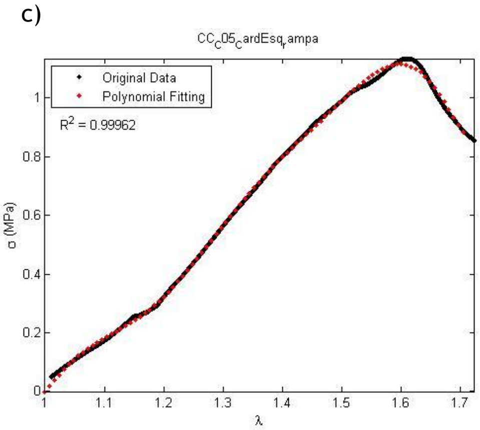
b)



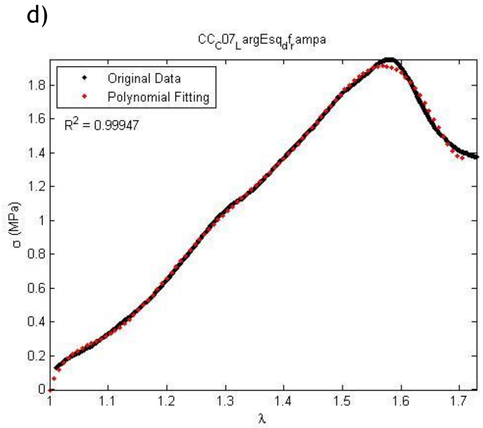
c)



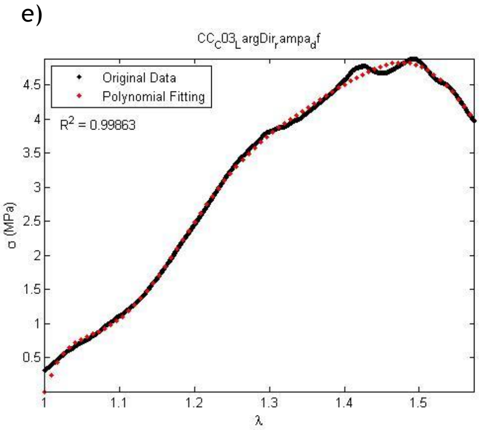
d)



e)



f)



g)

Figure B. 2 - Stress vs stretch curves for pre-menopausal specimens; Representation of polynomial fitting using SuperFit.m; a) Left Broad ligament from Case 03 ;b) Right Broad Ligament from Case 04 ;c) Right Round Ligaments from Case 04 ;d) Right Cardinal ligament from Case 05 ;e) Left Cardinal ligament from Case 05 ;f) Left Broad ligament from Case 07 ;g) Right Broad ligament from Case 03.

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